

#4

ENTERICALLY TRANSMITTED NON-A/NON-B HEPATITIS
VIRAL AGENT AND CHARACTERISTIC EPITOPES THEREOF



CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Application
Serial No. 08/279,823, filed July 25, 1994, which is a
continuation of U.S. Application Serial No. 07/681,078, filed
April 5, 1991, now abandoned, which is a continuation-in-part
10 of U.S. Application Serial No. 07/505,888, filed April 5, 1990,
now abandoned, which is a continuation-in-part of U.S.
Application Serial No. 07/420,921, filed October 13, 1989, now
abandoned, which is a continuation-in-part of U.S. Application
Serial No. 07/367,486, filed June 16, 1989, now abandoned,
15 which is a continuation-in-part of U.S. Application Serial No.
07/336,672, filed April 11, 1989, now abandoned, which is a
continuation-in-part of U.S. Application Serial No. 07/208,997,
filed June 17, 1988, now abandoned, all of which are herein
incorporated by reference.

20

INTRODUCTION

Field of Invention

This invention relates to recombinant proteins, genes,
and gene probes and more specifically to such proteins and
25 probes derived from an enterically transmitted nonA/nonB
hepatitis viral agent, to diagnostic methods and vaccine
applications which employ the proteins and probes, and to gene
segments that encode specific epitopes (and proteins
artificially produced to contain those epitopes) that are
30 particularly useful in diagnosis and prophylaxis.

Background

Enterically transmitted non-A/non-B hepatitis viral
agent (ET-NANB; also referred to herein as HEV) is the reported
35 cause of hepatitis in several epidemics and sporadic cases in
Asia, Africa, Europe, Mexico, and the Indian subcontinent.
Infection is usually by water contaminated with feces, although

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the virus may also spread by close physical contact. The virus does not seem to cause chronic infection. The viral etiology in ET-NANB has been demonstrated by infection of volunteers with pooled fecal isolates; immune electron microscopy (IEM) studies have shown virus particles with 27-34 nm diameters in stools from infected individuals. The virus particles reacted with antibodies in serum from infected individuals from geographically distinct regions, suggesting that a single viral agent or class is responsible for the majority of ET-NANB hepatitis seen worldwide. No antibody reaction was seen in serum from individuals infected with parenterally transmitted NANB virus (also known as hepatitis C virus or HCV), indicating a different specificity between the two NANB types.

In addition to serological differences, the two types of NANB infection show distinct clinical differences. ET-NANB is characteristically an acute infection, often associated with fever and arthralgia, and with portal inflammation and associated bile stasis in liver biopsy specimens (Arankalle). Symptoms are usually resolved within six weeks. Parenterally transmitted NANB, by contrast, produces a chronic infection in about 50% of the cases. Fever and arthralgia are rarely seen, and inflammation has a predominantly parenchymal distribution (Khuroo, 1980). The course of ET-NANBH is generally uneventful in healthy individuals, and the vast majority of those infected recover without the chronic sequelae seen with HCV. One peculiar epidemiologic feature of this disease, however, is the markedly high mortality observed in pregnant women; this is reported in numerous studies to be on the order of 10-20%. This finding has been seen in a number of epidemiologic studies but at present remains unexplained. Whether this reflects viral pathogenicity, the lethal consequence of the interaction of virus and immune suppressed (pregnant) host, or a reflection of the

debilitated prenatal health of a susceptible
malnourished population remains to be clarified.

The two viral agents can also be distin-
guished on the basis of primate host susceptibility.

5 ET-NANB, but not the parenterally transmitted agent,
can be transmitted to cynomolgus monkeys. The
parenterally transmitted agent is more readily
transmitted to chimpanzees than is ET-NANB (Bradley,
1987).

10 There have been major efforts worldwide to
identify and clone viral genomic sequences associated
with ET-NANB hepatitis. One goal of this effort,
requiring virus-specific genomic sequences, is to
15 identify and characterize the nature of the virus and
its protein products. Another goal is to produce
recombinant viral proteins which can be used in
antibody-based diagnostic procedures and for a
vaccine. Despite these efforts, viral sequences
20 associated with ET-NANB hepatitis have not been
successfully identified or cloned heretofore, nor have
any virus-specific proteins been identified or
produced.

Relevant Literature

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35 Khuroo, M.S., et al., Am. J. Med., 68:818
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Maniatis, T., et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1982).

Seto, B., et al., Lancet, 11:941 (1984).

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10 SUMMARY OF THE INVENTION

Novel compositions, as well as methods of preparation and use of the compositions are provided, where the compositions comprise viral proteins and fragments thereof derived from the viral agent for ET-
15 NANB. A number of specific fragments of viral proteins (and the corresponding genetic sequences) that are particularly useful in diagnosis and vaccine production are also disclosed. Methods for preparation of ET-NANB viral proteins include isolating ET-NANB
20 genomic sequences which are then cloned and expressed in a host cell. The resultant recombinant viral proteins find use as diagnostic agents and as vaccines. The genomic sequences and fragments thereof find use in preparing ET-NANB viral proteins and as
25 probes for virus detection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows vector constructions and manipulations used in obtaining and sequencing cloned
30 ET-NANB fragment; and

Figures 2A-2B are representations of Southern blots in which a radiolabeled ET-NANB probe was hybridized with amplified cDNA fragments prepared from RNA isolated from infected (I) and non-infected (N) bile sources (2A), and from infected (I) and non-
35 infected (N) stool-sample sources (2B).

DESCRIPTION OF SPECIFIC EMBODIMENTS

Novel compositions comprising generic sequences and fragments thereof derived from the viral agent for ET-NANB are provided, together with
5 recombinant viral proteins produced using the genomic sequences and methods of using these compositions. Epitopes on the viral protein have been identified that are particularly useful in diagnosis and vaccine production. Small peptides containing the epitopes are
10 recognized by multiple sera of patients infected with ET-NANB.

The molecular cloning of HEV was accomplished by two very different approaches. The first successful identification of a molecular clone was
15 based on the differential hybridization of putative HEV cDNA clones to heterogeneous cDNA from infected and uninfected cyno bile. cDNAs from both sources were labeled to high specific activity with ^{32}P to identify a clone that hybridized specifically to the
20 infected source probe. A cyno monkey infected with the Burma isolate of HEV was used in these first experiments. The sensitivity of this procedure is directly related to the relative abundance of the specific sequence against the overall background. In
25 control experiments, it was found that specific identification of a target sequence may be obtained with as little as 1 specific part per 1000 background sequences. A number of clones were identified by this procedure using libraries and probes made from
30 infected (Burma isolate) and control uninfected cyno bile. The first extensively characterized clone of the 16 plaques purified by this protocol was given the designation ET1.1.

ET1.1 was first characterized as both
35 derived from and unique to the infected source cDNA. Heterogeneous cDNA was amplified from both infected and uninfected sources using a sequence independent single premier amplification technique (SISPA). This

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technique is described in copending application serial
No. 208,512, filed June 17, 1988. The limited pool of
cDNA made from Burma infected cyno bile could then be
amplified enzymatically prior to cloning or
5 hybridization using putative HEV clones as probes.
ET1.1 hybridized specifically to the original bile
cDNA from the infected source. Further validation of
this clone as derived from the genome of HEV was
demonstrated by the similarity of the ET1.1 sequence
10 and those present in SISPA cDNA prepared from five
different human stool samples collected from
different ET-NANBH epidemics including Somalia,
Tashkent, Borneo, Mexico and Pakistan. These
molecular epidemiologic studies established the
15 isolated sequence as derived from the virus that
represented the major cause of ET-NANBH worldwide.

The viral specificity of ET1.1 was further
established by the finding that the clone hybridized
specifically to RNA extracted from infected cyno
20 liver. Hybridization analysis of polyadenylated RNA
demonstrated a unique 7.5 Kb polyadenylated
transcript not present in uninfected liver. The size
of this transcript suggested that it represented the
full length viral genome. Strand specific
25 oligonucleotides were also used to probe viral genomic
RNA extracted directly from semi-purified virions
prepared from human stool. The strand specificity was
based on the RNA-directed RNA polymerase (RDRP) open
reading frame (ORF) identified in ET1.1 (see below).
30 Only the probe detecting the sense strand hybridized
to the nucleic acid. These studies characterized HEV
as a plus sense, single stranded genome. Strand
specific hybridization to RNA extracted from the liver
also established that the vast majority of
35 intracellular transcript was positive sense. Barring
any novel mechanism for virus expression, the negative
strand, although not detectable, would be present at a

ratio of less than 1:100 when compared with the sense strand.

ET1.1 was documented as exogenous when tested by both Southern blot hybridization and PCR using genomic DNAs derived from uninfected humans, infected and uninfected cynos and also the genomic DNAs from E. coli and various bacteriophage sources. The latter were tested in order to rule out trivial contamination with an exogenous sequence introduced during the numerous enzymatic manipulations performed during cDNA construction and amplification. It was also found that the nucleotide sequence of the ET1.1 clone was not homologous to any entries in the Genbank database. The translated open reading frame of the ET1.1 clone did, however, demonstrate limited homology with consensus amino acid residues consistent with an RNA-directed RNA polymerase. This consensus amino acid motif is shared among all positive strand RNA viruses and, as noted above, is present at the 3' end of the HCV genome. The 1.3 Kb clone was therefore presumed to be derived, at least in part, from the nonstructural portion of the viral genome.

Because of the relationship of different strains of ET-NANB to each other that has been demonstrated by the present invention, the genome of the ET-NANB viral agent is defined in this specification as containing a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1 (ET1.1) carried in E. coli strain BB4 and having ATCC deposit no. 67717. The entire sequence, in both directions, has now been identified as set forth below. The sequences of both strands are provided, since both strands can encode proteins. However, the sequence in one direction has been designated as the "forward" sequence because of statistical similarities to known proteins and because the forward sequence is known to be predominately protein-encoding. This sequence is set forth below

along with the three possible translation sequences. There is one long open reading frame that starts at nucleotide 145 with an isoleucine and extends to the end of the sequence. The two other reading frames have many termination codons. Standard abbreviations for nucleotides and amino acids are used here and elsewhere in this specification.

The gene sequence given below is substantially identical to one given in the parent application. The present sequence differs in the omission of the first 37 nucleotides at the 5' end and last 13 nucleotides at the 3' end, which are derived from the linker used for cloning rather than from the virus. In addition, a G was omitted at position 227 of the sequence given in the parent application.

The following gene sequence has SEQ ID NO.1; the first amino acid sequence in reading frame beginning with nucleotide 1 has SEQ ID NO.2; the second amino acid sequence in reading frame beginning with nucleotide 2 has SEQ ID NO.3; and the third amino acid sequence in reading frame beginning with nucleotide 3 has SEQ ID NO.4.

Forward Sequence

SEQ ID NO. 1:

25	AGACCTGTCC CTGTTGCAGC TGTTCTACCA CCCTGCCCGG AGCTCGAACA GGGCCTTCTC	60
	TACCTGCCCC AGGAGCTCAC CACCTGTGAT AGTGTCTGTA CATTGAATT AACAGACATT	120
30	GTGCACTGCC GCATGGCCGC CCCGAGCCAG CGCAAGGCCG TGCTGTCCAC ACTCGTGGGC	180
	CGCTACGGCG GTCGCACAAA GCTCTACAAT GCTTCCCACT CTGATGTTTCG CGACTCTCTC	240
	GCCCCGTTTTA TCCCGGCCAT TGGCCCCGTA CAGGTTACAA CTTGTGAATT GTACGAGCTA	300
35	GTGGAGGCCA TGGTCGAGAA GGGCCAGGAT GGCTCCGCCG TCCTTGAGCT TGATCTTTGC	360
	AACCGTGACG TGTCAGGAT CACCTTCTTC CAGAAAGATT GTAACAAGTT CACCACAGGT	420
40	GAGACCATTG CCCATGGTAA AGTGGGCCAG GGCATCTCGG CCTGGAGCAA GACCTTCTGC	480
	GCCCTCTTTG GCCCTTGTTT CCGCGCTATT GAGAAGGCTA TTCTGGCCCT GCTCCCTCAG	540
45	GGTGTGTTTT ACGGTGATGC CTTTGATGAC ACCGTCTTCT CGGCGGCTGT GGCCGCAGCA	600

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AAGGCATCCA TGGTGTTTGA GAATGACTTT TCTGAGTTTG ACTCCACCCA GAATACTTT 660
TCTCTGGGTC TAGAGTGTGC TATTATGGAG GAGTGTGGGA TGCCGCAGTG GCTCATCCGC 720
5 CTGTATCACC TTATAAGGTC TCGTGGATC TTGCAGGCC CGAAGGAGTC TCTGCGAGGG 780
TTTTGGAAGA AACACTCCGG TGAGCCCGGC ACTCTTCTAT GGAATACTGT CTGGAATATG 840
GCCGTTATTA CCCACTGTGA TGAATCCGC GATTTTCAGG TGGCTGCCTT TAAAGGTGAT 900
10 GATTCGATAG TGCTTTGCAG TGAGTATCGT CAGAGTCCAG GAGCTGCTGT CCTGATCGCC 960
GGCTGTGGCT TGAAGTTGAA GGTAGATTTC CGCCCGATCG GTTTGTATGC AGGTGTTGTG 1020
15 GTGGCCCCCG GCCTTGGCGC GCTCCCTGAT GTTGTGCGCT TCGCCGGCCG GCTTACCGAG 1080
AAGAATTGGG GCCCTGGCCC TGAGCGGGCG GAGCAGCTCC GCCTCGCTGT TAGTGATTTT 1140
CTCCGCAAGC TCACGAATGT AGCTCAGATG TGTGTGGATG TTGTTTCCCG TGTTTATGGG 1200
20 GTTCCCTG GACTCGTTCA TAACCTGATT GGCATGCTAC AGGCTGTTGC TGATGGCAAG 1260
GCACATTTC CTGAGTCAGT AAAACCTAGT CTCGA 1295

25 SEQ ID NO. 2:

Arg Pro Val Pro Val Ala Ala Val Leu Pro Pro Cys Pro Glu Leu Glu
1 5 10 15
30 Gln Gly Leu Leu Tyr Leu Pro Gln Glu Leu Thr Thr Cys Asp Ser Val
20 25 30
Val Thr Phe Glu Leu Thr Asp Ile Val His Cys Arg Met Ala Ala Pro
35 40 45
35 Ser Gln Arg Lys Ala Val Leu Ser Thr Leu Val Gly Arg Tyr Gly Gly
50 55 60
40 Arg Thr Lys Leu Tyr Asn Ala Ser His Ser Asp Val Arg Asp Ser Leu
65 70 75 80
Ala Arg Phe Ile Pro Ala Ile Gly Pro Val Gln Val Thr Thr Cys Glu
85 90 95
45 Leu Tyr Glu Leu Val Glu Ala Met Val Glu Lys Gly Gln Asp Gly Ser
100 105 110
Ala Val Leu Glu Leu Asp Leu Cys Asn Arg Asp Val Ser Arg Ile Thr
115 120 125
50 Phe Phe Gln Lys Asp Cys Asn Lys Phe Thr Thr Gly Glu Thr Ile Ala
130 135 140
55 His Gly Lys Val Gly Gln Gly Ile Ser Ala Trp Ser Lys Thr Phe Cys
145 150 155 160

	Ala	Leu	Phe	Gly	Pro	Trp	Phe	Arg	Ala	Ile	Glu	Lys	Ala	Ile	Leu	Ala	
					165						170					175	
5	Leu	Leu	Pro	Gln	Gly	Val	Phe	Tyr	Gly	Asp	Ala	Phe	Asp	Asp	Thr	Val	
				180					185						190		
	Phe	Ser	Ala	Ala	Val	Ala	Ala	Ala	Lys	Ala	Ser	Met	Val	Phe	Glu	Asn	
			195						200				205				
10	Asp	Phe	Ser	Glu	Phe	Asp	Ser	Thr	Gln	Asn	Asn	Phe	Ser	Leu	Gly	Leu	
		210					215					220					
	Glu	Cys	Ala	Ile	Met	Glu	Glu	Cys	Gly	Met	Pro	Gln	Trp	Leu	Ile	Arg	
	225					230					235					240	
15	Leu	Tyr	His	Leu	Ile	Arg	Ser	Ala	Trp	Ile	Leu	Gln	Ala	Pro	Lys	Glu	
				245						250						255	
	Ser	Leu	Arg	Gly	Phe	Trp	Lys	Lys	His	Ser	Gly	Glu	Pro	Gly	Thr	Leu	
20				260					265						270		
	Leu	Trp	Asn	Thr	Val	Trp	Asn	Met	Ala	Val	Ile	Thr	His	Cys	Tyr	Asp	
			275					280						285			
25	Phe	Arg	Asp	Phe	Gln	Val	Ala	Ala	Phe	Lys	Gly	Asp	Asp	Ser	Ile	Val	
		290					295					300					
	Leu	Cys	Ser	Glu	Tyr	Arg	Gln	Ser	Pro	Gly	Ala	Ala	Val	Leu	Ile	Ala	
	305					310					315					320	
30	Gly	Cys	Gly	Leu	Lys	Leu	Lys	Val	Asp	Phe	Arg	Pro	Ile	Gly	Leu	Tyr	
				325						330					335		
	Ala	Gly	Val	Val	Val	Ala	Pro	Gly	Leu	Gly	Ala	Leu	Pro	Asp	Val	Val	
35				340					345					350			
	Arg	Phe	Ala	Gly	Arg	Leu	Thr	Glu	Lys	Asn	Trp	Gly	Pro	Gly	Pro	Glu	
			355					360						365			
40	Arg	Ala	Glu	Gln	Leu	Arg	Leu	Ala	Val	Ser	Asp	Phe	Leu	Arg	Lys	Leu	
		370					375					380					
	Thr	Asn	Val	Ala	Gln	Met	Cys	Val	Asp	Val	Val	Ser	Arg	Val	Tyr	Gly	
	385					390					395					400	
45	Val	Ser	Pro	Gly	Leu	Val	His	Asn	Leu	Ile	Gly	Met	Leu	Gln	Ala	Val	
				405						410					415		
	Ala	Asp	Gly	Lys	Ala	His	Phe	Thr	Glu	Ser	Val	Lys	Pro	Val	Leu		
50				420					425					430			

SEQ ID NO. 3:

Asp Leu Ser Leu Leu Gln Leu Phe Tyr His Pro Ala Pro Ser Ser Asn
55 1 5 10 15

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	Arg Ala Phe Ser Thr Cys Pro Arg Ser Ser Pro Pro Val Ile Val Ser	20	25	30
5	. His Leu Asn . Gln Thr Leu Cys Thr Ala Ala Trp Pro Pro Arg	35	40	45
	Ala Ser Ala Arg Pro Cys Cys Pro His Ser Trp Ala Ala Thr Ala Val	50	55	60
10	Ala Gln Ser Ser Thr Met Leu Pro Thr Leu Met Phe Ala Thr Leu Ser	65	70	75
	Pro Val Leu Ser Arg Pro Leu Ala Pro Tyr Arg Leu Gln Leu Val Asn	85	90	95
15	Cys Thr Ser . Trp Arg Pro Trp Ser Arg Arg Ala Arg Met Ala Pro	100	105	110
	Pro Ser Leu Ser Leu Ile Phe Ala Thr Val Thr Cys Pro Gly Ser Pro	115	120	125
20	Ser Ser Arg Lys Ile Val Thr Ser Ser Pro Gln Val Arg Pro Leu Pro	130	135	140
	Met Val Lys Trp Ala Arg Ala Ser Arg Pro Gly Ala Arg Pro Ser Ala	145	150	155
25	Pro Ser Leu Ala Leu Gly Ser Ala Leu Leu Arg Arg Leu Phe Trp Pro	165	170	175
30	Cys Ser Leu Arg Val Cys Phe Thr Val Met Pro Leu Met Thr Pro Ser	180	185	190
	Ser Arg Arg Leu Trp Pro Gln Gln Arg His Pro Trp Cys Leu Arg Met	195	200	205
35	Thr Phe Leu Ser Leu Thr Pro Pro Arg Ile Thr Phe Leu Trp Val .	210	215	220
	Ser Val Leu Leu Trp Arg Ser Val Gly Cys Arg Ser Gly Ser Ser Ala	225	230	235
40	Cys Ile Thr Leu . Gly Leu Arg Gly Ser Cys Arg Pro Arg Arg Ser	245	250	255
45	Leu Cys Glu Gly Phe Gly Arg Asn Thr Pro Val Ser Pro Ala Leu Phe	260	265	270
	Tyr Gly Ile Leu Ser Gly Ile Trp Pro Leu Leu Pro Thr Val Met Thr	275	280	285
50	Ser Ala Ile Phe Arg Trp Leu Pro Leu Lys Val Met Ile Arg . Cys	290	295	300
	Phe Ala Val Ser Ile Val Arg Val Gln Glu Leu Leu Ser . Ser Pro	305	310	315
55				320

Ala Val Ala . Ser . Arg . Ile Ser Ala Arg Ser Val Cys Met
325 330 335

5 Gln Val Leu Trp Trp Pro Pro Ala Leu Ala Arg Ser Leu Met Leu Cys
340 345 350

Ala Ser Pro Ala Gly Leu Pro Arg Arg Ile Gly Ala Leu Ala Leu Ser
355 360 365

10 Gly Arg Ser Ser Ser Ala Ser Leu Leu Val Ile Ser Ser Ala Ser Ser
370 375 380

Arg Met . Leu Arg Cys Val Trp Met Leu Phe Pro Val Phe Met Gly
385 390 395 400

Phe Pro Leu Asn Ser Phe Ile Thr . Leu Ala Cys Tyr Arg Leu Leu
405 410 415

20 Leu Met Ala Arg His Ile Ser Leu Ser Gln . Asn Gln Cys Ser
420 425 430

SEQ ID NO. 4:

25 Thr Cys Pro Cys Cys Ser Cys Ser Thr Thr Leu Pro Arg Ala Arg Thr
1 5 10 15

Gly Pro Ser Leu Pro Ala Pro Gly Ala His His Leu . . Cys Arg
20 25 30

30 Asn Ile . Ile Asn Arg His Cys Ala Leu Pro His Gly Arg Pro Glu
35 40 45

35 Pro Ala Gln Gly Arg Ala Val His Thr Arg Gly Pro Leu Arg Arg Ser
50 55 60

His Lys Ala Leu Gln Cys Phe Pro Leu . Cys Ser Arg Leu Ser Arg
65 70 75 80

40 Pro Phe Tyr Pro Gly His Trp Pro Arg Thr Gly Tyr Asn Leu . Ile
85 90 95

Val Arg Ala Ser Gly Gly His Gly Arg Glu Gly Pro Gly Trp Leu Arg
100 105 110

45 Arg Pro . Ala . Ser Leu Gln Pro . Arg Val Gln Asp His Leu
115 120 125

Leu Pro Glu Arg Leu . Gln Val His His Arg . Asp His Cys Pro
130 135 140

50 Trp . Ser Gly Pro Gly His Leu Gly Leu Glu Gln Asp Leu Leu Arg
145 150 155 160

55 Pro Leu Trp Pro Leu Val Pro Arg Tyr . Glu Gly Tyr Ser Gly Pro
165 170 175

Ala Pro Ser Gly Cys Val Leu Arg . Cys Leu . . His Arg Leu
 180 185 190
 5 Leu Gly Gly Cys Gly Arg Ser Lys Gly Ile His Gly Val . Glu .
 195 200 205
 Leu Phe . Val . Leu His Pro Glu . Leu Phe Ser Gly Ser Arg
 210 215 220
 10 Val Cys Tyr Tyr Gly Gly Val Trp Asp Ala Ala Val Ala His Pro Pro
 225 230 235 240
 Val Ser Pro Tyr Lys Val Cys Val Asp Leu Ala Gly Pro Glu Gly Val
 15 245 250 255
 Ser Ala Arg Val Leu Glu Glu Thr Leu Arg . Ala Arg His Ser Ser
 260 265 270
 20 Met Glu Tyr Cys Leu Glu Tyr Gly Arg Tyr Tyr Pro Leu Leu . Leu
 275 280 285
 Pro Arg Phe Ser Gly Gly Cys Leu . Arg . . Phe Asp Ser Ala
 290 295 300
 25 Leu Gln . Val Ser Ser Glu Ser Arg Ser Cys Cys Pro Asp Arg Arg
 305 310 315 320
 Leu Trp Leu Glu Val Glu Gly Arg Phe Pro Pro Asp Arg Phe Val Cys
 30 325 330 335
 Arg Cys Cys Gly Gly Pro Arg Pro Trp Arg Ala Pro . Cys Cys Ala
 340 345 350
 35 Leu Arg Arg Pro Ala Tyr Arg Glu Glu Leu Gly Pro Trp Pro . Ala
 355 360 365
 Gly Gly Ala Ala Pro Pro Arg Cys . . Phe Pro Pro Gln Ala His
 370 375 380
 40 Glu Cys Ser Ser Asp Val Cys Gly Cys Cys Phe Pro Cys Leu Trp Gly
 385 390 395 400
 Phe Pro Trp Thr Arg Ser . Pro Asp Trp His Ala Thr Gly Cys Cys
 45 405 410 415
 . Trp Gln Gly Thr Phe His . Val Ser Lys Thr Ser Ala Arg
 420 425 430

50 The complementary strand, referred to here
 as the "reverse sequence," is set forth below in the
 same manner as the forward sequence set forth above.
 Several open reading frames, shorter than the long
 open reading frame found in the forward sequence, can

be seen in this reverse sequence. Because of the relative brevity of the open reading frames in the reverse direction, they are probably not expressed.

The following gene sequence has SEQ ID NO.5.

5 Reverse Sequence

SEQ ID NO. 5:

	TCGAGCACTG GTTTTACTGA CTCAGTGAAA TGTGCCTTGC CATCAGCAAC AGCCTGTAGC	60
10	ATGCCAATCA GGTATGAAC GAGTCCAGGG GAAACCCCAT AAACACGGGA AACAACTCC	120
	ACACACATCT GAGCTACATT CGTGAGCTTG CGGAGGAAAT CACTAACAGC GAGGCGGAGC	180
	TGCTCCGCCC GCTCAGGGCC AGGGCCCCAA TTCTTCTCGG TAAGCCGGCC GGCGAAGCGC	240
15	ACAACATCAG GGAGCGCGCC AAGGCCGGGG GCCACCACAA CACCTGCATA CAAACCGATC	300
	GGGCGGAAAT CTACCTTCAA CTTCAAGCCA CAGCCGGCGA TCAGGACAGC AGCTCCTGGA	360
20	CTCTGACGAT ACTCACTGCA AAGCACTATC GAATCATCAC CTTTAAAGGC AGCCACCTGA	420
	AAATCGCGGA AGTCATAACA GTGGGTAATA ACGGCCATAT TCCAGACAGT ATTCCATAGA	480
	AGAGTGCCGG GCTCACCGBA GTGTTTCTTC CAAAACCTC GCAGAGACTC TTTCGGGGCC	540
25	TGCAAGATCC ACGCAGACCT TATAAGGTGA TACAGGCGGA TGAGCCACTG CGGCATCCCA	600
	CACTCCTCCA TAATAGCACA CTCTAGACCC AGAGAAAAGT TATTCTGGGT GGAGTCAAAC	660
30	TCAGAAAAGT CATTCTCAA CACCATGGAT GCTTTGCTG CGGCCACAGC CGCCGAGAAG	720
	ACGGTGTGAT CAAAGGCATC ACCGTAAAC ACACCCTGAG GGAGCAGGGC CAGAATAGCC	780
	TTCTCAATAG CGCGGAACCA AGGGCCAAAG AGGGCGCAGA AGGTCTTGCT CCAGGCCGAG	840
35	ATGCCCTGGC CCACTTTACC ATGGGCAATG GTCTCACCTG TGGTGAAGTT GTTACAATCT	900
	TTCTGGAAGA AGGTGATCCT GGACACGTCA CGGTTGCAA GATCAAGCTC AAGGACGGCG	960
40	GAGCCATCCT GGCCCTTCTC GACCATGGCC TCCACTAGCT CGTACAATTC ACAAGTTGTA	1020
	ACCTGTACGG GGCCAATGGC CGGGATAAAA CGGGCGAGAG AGTCGCGAAC ATCAGAGTGG	1080
	GAAGCATTGT AGAGCTTTGT GCGACCGCCG TAGCGGCCCA CGAGTGTGGA CAGCACGGCC	1140
45	TTGCGCTGGC TCGGGCGGGC CATGCGGCAG TGCACAATGT CTGTAAATTC AAATGTTACG	1200
	ACACTATCAC AGGTGGTGAG CTCCTGGGGC AGGTAGAGAA GGCCCTGTTC GAGCTCGGGG	1260
50	CAGGGTGGTA GAACAGCTGC AACAGGGACA GGTCT	1295

Identity of this sequence with sequences in etiologic agents has been confirmed by locating a

N P F C G E S T L Y T R T W S E V D A V
 AATCCATTCTGTGGCGAGAGCACACTTTACACCCGTACTTGGTCGGAGGTTGATGCCGTC 2160
 5 S S P A R P D L G F M S E P S I P S R A
 TCTAGTCCAGCCCGGCTGACTTAGGTTTTATGTCTGAGCCTTCTATACCTAGTAGGGCC
 A T P T L A A P L P P P A P D P S P P P
 GCCACGCCTACCCCTGGCGGCCCTCTACCCCCCTGCACCGGACCCTTCCCCCTCC 2280
 10 S A P A L A E P A S G A T A G A P A I T
 TCTGCCCCGGCGCTTGCTGAGCCGGCTTCTGGCGCTACCGCCGGGGCCCCGGCCATACT
 H Q T A R H R R L L F T Y P D G S K V F
 CACCAGACGGCCCGGCACCGCCGCTGCTCTTACCTACCCGGATGGCTCTAAGGTATTC 2400
 15 A G S L F E S T C T W L V N A S N V D H
 GCCGGCTCGCTGTTGAGTCGACATGCACGTGGCTCGTTAACGCGTCTAATGTTGACCAC
 R P G G G L C H A F Y Q R Y P A S F D A
 CGCCCTGGCGGGGGCTTTGCCATGCATTTTACCAAAGGTACCCCGCTCCTTTGATGCT 2520
 20 A S F V M R D G A A A Y T L T P R P I I
 GCCTCTTTTGATGCGCGACGGCGCGGCGGTACACACTAACCCCCGGCCAATAATT
 H A V A P D Y R L E H N P K R L E A A Y
 CACGCTGTCGCCCTGATTATAGGTTGGAACATAACCCAAAGAGGCTTGAGGCTGCTTAT 2640
 25 R E T C S R L G T A A Y P L L G T G I Y
 CGGGAAACTTGCTCCCGCTCGGCACCGCTGCATACCCGCTCCTCGGACCGGCATATAC
 30 Q V P I G P S F D A W E R N H R P G D E
 CAGGTGCCGATCGGCCAGTTTGTGACGCTGGGAGCGGAACACCGCCCCGGGGATGAG 2760
 35 L Y L P E L A A R W F E A N R P T R P T
 TTGTACCTTCTGAGCTTGCTGCCAGATGGTTTGAGGCCAATAGGCCGACCCGCCGACT
 L T I T E D V A R T A N L A I E L D S A
 CTCCTATAACTGAGGATGTTGCACGGACAGCAATCTGGCCATCGAGCTTGACTCAGCC 2880
 40 T D V G R A C A G C R V T P G V V Q Y Q
 ACAGATGTCGGCCGGGCTGTGCCGGCTGTCGGGTACCCCGGCGTTGTTCACTACAG
 F T A G V P G S G K S R S I T Q A D V D
 TTTACTGCAGGTGTGCTGGATCCGGCAAGTCCCGCTCTATACCCAAGCCGATGTGGAC 3000
 45 V V V V P T R E L R N A W R R R G F A A
 GTTGTCTGGTCCCAGCGGTGAGTTGCGTAATGCTGGCGCGCTCGCGGCTTTGCTGCT
 F T P H T A A R V T Q G R R V V I D E A
 TTTACCCGCATACTGCCGCCAGAGTCACCCAGGGGCGCCGGTTGTCTTGTGATGAGGCT 3120
 50 P S L P P H L L L L H M Q R A A T V H L
 CCATCCCTCCCCCTCACTGCTGCTGCTCCACATGCAGCGGGCCGCCACCGTCCACCTT
 L G D P N Q I P A I D F E H A G L V P A
 CTTGGCGACCCGAACCAGATCCCAGCCATCGACTTTGAGCACGCTGGGCTCGTCCCCGCC 3240
 55 I R P D L G P T S W W H V T H R W P A D
 ATCAGGCCGACTTAGGCCCCACCTCCTGGTGGCATGTTACCCATCGCTGGCCTGCGGAT
 60

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V C E L I R G A Y P M I Q T T S R V L R
GTATGCGAGCTCATCCGTGGTGCATACCCATGATCCAGACCTAGCCGGTTCTCCGT 3360
S L F W G E P A V G Q K L V F T Q A A K
5 TCGTTGTTCTGGGGTGAGCCTGCCGTGGGGCAGAACTAGTGTTACCCAGGCGGCCAAG
P A N P G S V T V H E A Q G A T Y T E T
CCCGCCAACCCGGCTCAGTGACGGTCCACGAGGCGAGGGCGCTACCTACACGGAGACC 3480
10 T I I A T A D A R G L I Q S S R A H A I
ACTATTATTGCCACAGCAGATGCCCCGGGGCCTTATTCAGTCGTCTCGGGCTCATGCCATT
V A L T R H T E K C V I I D A P G L L R
GTTGCTCTGACGCGCCACACTGAGAAGTGCCTCATCATTGACGCACCAAGGCTGCTTCGC 3600
15 E V G I S D A I V N N F F L A G G E I G
GAGGTGGGCATCTCCGATGCAATCGTTAATAACTTTTTCTCGCTGGTGGCGAAATTGGT
H Q R P S V I P R G N P D A N V D T L A
20 CACCAGCGCCCATCAGTTATTCCCCGTGGCAACCCTGACGCCAATGTTGACACCCTGGCT 3720
A F P P S C Q I S A F H Q L A E E L G H
GCCTTCCCGCGCTCTTGCCAGATTAGTGCTTCCATCAGTTGGCTGAGGAGCTTGGCCAC
25 R P V P V A A V L P P C P E L E Q G L L
AGACCTGTCCCTGTTGACGTGTTCTACCACCCTGCCCGAGCTCGAACAGGGCCTTCTC 3840
Y L P Q E L T T C D S V V T F E L T D I
TACCTGCCCCAGGAGCTCACCACCTGTGATAGTGTGTAACATTGGAATTAACAGACATT
30 V H C R M A A P S Q R K A V L S T L V G
GTGCACTGCCGATGGCCGCCCCGAGCCAGCGCAAGGCCGTGCTGTCCACACTCGTGGGC 3960
R Y G G R T K L Y N A S H S D V R D S L
35 CGCTACGGCGGTGCGACAAAGCTCTACAATGCTTCCCACTCTGATGTTGCGGACTCTCT
A R F I P A I G P V Q V T T C E L Y E L
GCCCCGTTTTATCCCGCCATTGGCCCCGTACAGGTTACAACCTGTGAATTGTACGAGCTA 4080
40 V E A M V E K G Q D G S A V L E L D L C
GTGGAGGCCATGGTGCAGAAGGGCCAGGATGGCTCCGCCGTCTTGAGCTTGATCTTTGC
N R D V S R I T F F Q K D C N K F T T G
45 AACCGTGACGTGTCCAGGATCACCTTCTTCCAGAAAGATTGTAACAAGTTACCACAGGT 4200
E T I A H G K V G Q G I S A S K T F C
GAGACCATTGCCCATGGTAAAGTGGGCCAGGGCATCTCGGCCTGGAGCAAGACCTTCTGC
A L F G P W F R A I E K A I L A L L P Q
50 GCCCTCTTTGGCCCTTGGTTCGCGCTATTGAGAAGGCTATTCTGGCCCTGCTCCCTCAG 4320
G V F Y G D A F D D T V F S A A V A A A
GGTGTGTTTTACGGTGATGCCTTTGATGACACCGTCTTCTCGGCGGTGTGGCCGCGAGCA
K A S M V F E N D F S E F D S T Q N N F
55 AAGGCATCCATGGTGTGAGAATGACTTTTCTGAGTTTGACTCCACCCAGAATAACTTT 4440
S L G L E C A I M E E C G M P Q W L I R
TCTCTGGGCTAGAGTGTGCTATTATGGAGGAGTGTGGGATGCCGCAGTGGCTCATCCGC
60

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LYHLIRSAWILQAPKESLRG
CTGTATCACCTTATAAGGTCTGCGTGGATCTTGAGGCCCGAAGGAGTCTCTGCGAGGG 4560
FWKKHSGEPGTLWLNTVWNM
5 TTTTGAAGAACTCCGGTGAGCCCGGCACTCTTCTATGGAATACTGTCTGGAATATG
AVITHCYDFRDFQVAAFKG
GGCGTTATTACCACTGTTATGACTTCGCGATTTTCAGGTGGCTGCCTTTAAAGGTGAT 4680
10 DSIVLCSEYRQSPGA AVLIA
GATTCGATAGTGCTTTCAGTGAGTATCGTCAGAGTCCAGGAGCTGCTGTCTGATCGCC
GCGGLKLKVDFRPIGLYAGVV
GGCTGTGGCTGAAGTTGAAGGTAGATTTCCGCCGATCGGTTTGTATGAGGTGTTGTG 4800
15 VAPGLGALPDVVRFA GRLTE
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KNWGP GPERAEQLRLAVSDF
20 AAGAATTGGGGCCCTGGCCCTGAGCGGGGAGCAGCTCCGCCCTCGCTGTTAGTGATTTTC 4920
LRKLTNV AQMCVDVVS RVY G
CTCCGCAAGCTCACAATGTAGCTCAGATGTGTGGATGTTGTTTCCCGTGTTTATGGG
25 VSPGLVHNLIGMLQAVADGK
GTTTCCCTGGACTCGTTCAACCTGATTGGCATGCTACAGGCTGTTGCTGATGGCAAG 5040
AHFTESVKPVLDLTNSILCR
30 GCACATTTCACTGAGTCAGTAAACAGTGCTCGACTTGACAAATCAATCTGTGTGCGG
|-ORF3--->
MNNMSFAAPMGSRPCALG
MRPRP
35 VEZ
GTGGAATGAATAACATGTCTTTTGCTGCGCCCATGGGTTTCGCGACCATGCGCCCTCGGCC 5160
LFCSSSCFC LCCPRHRPV S
40 ILLLLLMFLPMLPAPPPGP
TATTTTGTGCTGCTCCTCATGTTTTTGCTATGCTGCCCGCGCCACCGCCCGGTACGCC
RLAAVVVGGA AVPAVVSGVT
45 SGRRRGRRS GGGSGGGGFWGDR
GTCTGGCCGCGTCTGTTGGGCGGCGAGCGGCGGTTCCGGCGGTGTTTCTGGGGTGACCG 5280
GLILSPSQSPIFIQPTPSPP
50 VDSQPFAIPYIHP TNPFA PD
GGTTGATTCTCAGCCCTTCGCAATCCCTATATTATCAACCAACCCCTTCGCCCCCGA
MSPLRPGLDLVFANPPDHS A
55 VTAAGAAGPRVRQPAPPLGS
TGTCACCGCTGCGGCCGGGGCTGGACCTCGTGTTCGCCAACCGCCCGACCACTCGGCTC 5400

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P L G V T R P S A P P L P H V V D L P Q
A W R D Q A Q R P A V A S R R R P T T A
CGCTTGGCGTGACCAGGCCAGCGCCCCGCCGTTGCCTCACGTCGTAGACCTACCACAGC
5
L G P R R Z
G A A P L T A V A P A H D T P P V P D V
TGGGGCCGCGCGCTAACCGCGGTCGCTCCGGCCCATGACACCCGCCAGTGCCTGATGT 5520
10
D S R G A I L R R Q Y N L S T S P L T S
CGACTCCCGCGGCGCCATCTTGCGCCGGCAGTATAACCTATCAACATCTCCCCTTACCTC
15
S V A T G T N L V L Y A A P L S P L L P
TTCCGTGGCCACCGGCACTAACCTGGTTCTTTATGCCGCCCTCTTAGTCCGCTTTTACC 5640
20
L Q D G T N T H I M A T E A S N Y A Q Y
CCTTCAGGACGGCACCAATACCCATATAATGGCCACGGAAGCTTCTAATTATGCCCAGTA
R V A R A T I R Y R P L V P N A V G G Y
25
CCGGGTTGCCGTGCCACAATCCGTTACCGCCCGCTGGTCCCCAATGCTGTGCGCGGTTA 5760
A I S I S F W P Q T T T T P T S V D M N
CGCCATCTCCATCTCATTCTGGCCACAGACCACCACCCCGACGTCGGTTGATATGAA
30
S I T S T D V R I L V Q P G I A S E L V
TTCAATAACCTCGACGGATGTTTCGTATTTTAGTCCAGCCCGGCATAGCCTCTGAGCTTGT 5880
35
I P S E R L H Y R N Q G W R S V E T S G
GATCCCAAGTGAGCGCCTACACTATCGTAACCAAGGCTGGCGCTCCGTCGAGACCTCTGG
V A E E E A T S G L V M L C I H G S L V
40
GGTGGCTGAGGAGGAGGCTACCTCTGGTCTTGTTATGCTTTGCATACATGGCTCACTCGT 6000
N S Y T N T P Y T G A L G L L D F A L E
45
AAATTCCTATACTAATACACCTATACCGGTGCCCTCGGGCTGTTGGACTTTGCCCTTGA
L E F R N L T P G N T N T R V S R Y S S
GCTTGAGTTTCGCAACCTTACCCCGGTAACACCAATACGCGGGTCTCCCGTTATTCCAG 6120
50
T A R H R L R R G A D G T A E L T T T A
CACTGCTCGCCACCGCCTTCGTCGCGGTGCGGACGGGACTGCCGAGCTCACCACCACGGC
55
A T R F M K D L Y F T S T N G V G E I G
TGCTACCCGCTTTATGAAGGACCTCTATTTTACTAGTACTAATGGTGTCGGTGAGATCGG 6240

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R G I A L T L F N L A D T L L G G L P T
CCGCGGGATAGCCCTCACCTGTTCAACCTTGCTGACACTCTGCTTGGCGGCCTGCCGAC
5 E L I S S A G G Q L F Y S R P V V S A N
AGAATTGATTTTCGTGGCTGGTGGCCAGCTGTTCTACTCCCGTCCCGTTGTCTCAGCCAA 6360
10 G E P T V K L Y T S V E N A Q Q D K G I
TGGCGAGCCGACTGTTAAGTTGTATACATCTGTAGAGAATGCTCAGCAGGATAAGGGTAT
A I P H D I D L G E S R V V I Q D Y D N
15 TGCAATCCCGCATGACATTGACCTCGGAGAATCTCGTGTGGTTATTCAGGATTATGATAA 6480
Q H E Q D R P T P S P A P S R P F S V L
CCAACATGAACAAGATCGGCCGACGCCCTTCTCCAGCCCCATCGCGCCCTTTCTCTGTCTCT
20 R A N D V L W L S L T A A E Y D Q S T Y
TCGAGCTAATGATGTGCTTTGGCTCTCTCTCACCCTGCGGAGTATGACCAGTCCACTTA 6600
25 G S S T G P V Y V S D S V T L V N V A T
TGGCTCTTCGACTGGCCAGTTTATGTTTCTGACTCTGTGACCTTGGTTAATGTTGCGAC
G A Q A V A R S L D W T K V T L D G R P
30 CGGCGCGCAGGCCGTTGCCCGGTGCTCGATTGGACCAAGGTCACACTTGACGGTCGCCC 6720
L S T I Q Q Y S K T F F V L P L R G K L
35 CCTCTCCACCATCCAGCAGTACTCGAAGACCTTCTTTGTCCTGCGCTCCGCGGTAAGCT
S F W E A G T T K A G Y P Y N Y N T T A
CTCTTTCTGGGAGGCAGGCACAATAAGCCGGGTACCCTTATAATTATAACACCACTGC 6840
40 S D Q L L V E N A A G H R V A I S T Y T
TAGCGACCAACTGCTTGTGAGAATGCCGCCGGGCACCGGGTCGCTATTTCCACTTACAC
45 T S L G A G P V S I S A V A V L A P H S
CACTAGCCTGGGTGCTGGTCCCGTCTCCATTTCTGCGGTTGCCGTTTATAGCCCCCACTC 6960
A L A L L E D T L D Y P A R A H T F D D
50 TGCCTAGCATTGCTTGAGGATACCTTGGACTACCCTGCCCGCGCCATACTTTTGATGA
F C P E C R P L G L Q G C A F Q S T V A
55 TTTCTGCCAGAGTGCCGCCCTTGGCCTTCAGGGCTGCGCTTTCCAGTCTACTGTGCG 7080
E L Q R L K M K V G K T R E L Z
60 TGAGCTTCAGCGCCTTAAGATGAAGGTGGGTAAACTCGGGAGTTGTAGTTTATTGCTT

5 Total number of bases in this sequence as presented is 7195. The poly-A tail present in the cloned sequence has been omitted.

10 The ability of the methods described herein to isolate and identify genetic material from other NANB hepatitis strains has been confirmed by identifying genetic material from an isolate obtained in Mexico. The sequence of this isolate was about 75% identical to the ET1.1 sequence set forth in SEQ ID NO.1 above. The sequence was identified by hybridization using the conditions set forth in Section II.B below.

15 In this different approach to isolation of the virus, cDNA libraries were made directly from a semi-purified human stool specimen collected from an outbreak of ET-NANB in Telixtac. The recovery of cDNA and the construction of representative libraries was assured by the application of sequence independent
20 single premier amplification (SISPA). A cDNA library constructed in lambda gtl1 from such an amplified cDNA population was screened with a serum considered to have "high" titer anti-HEV antibodies as assayed by direct immunofluorescence on liver sections from
25 infected cynos. Two cDNA clones, denoted 406.3-2 and 406.4-2, were identified by this approach from a total of 60,000 screened. The sequence of these clones was subsequently localized to the 3' half of the viral genome by homology comparison to the HEV (Burma)
30 sequence obtained from clones isolated by hybridization screening of libraries with the original ET1.1 clone.

35 These isolated cDNA epitopes when used as hybridization probes on Northern blots of RNA extracted from infected cyno liver gave a somewhat different result when compared to the Northern blots obtained with the ET1.1 probe. In addition to the single 7.5 Kb transcript seen using ET1.1, two

additional transcripts of 3.7 and 2.0 Kb were identified using either of these epitopes as hybridization probes. These polyadenylated transcripts were identified using the extreme 3' end epitope clone (406.3-2) as probe and therefore established these transcripts as co-terminal with the 3' end of the genome (see below). One of the epitope clones (406.4-2) was subsequently shown to react in a specific fashion with antisera collected from 5 different geographic epidemics (Somalia, Burma, Mexico, Tashkent and Pakistan). The 406.3-2 clone reacted with sera from 4 out of these same 5 epidemics (Yarborough et al., 1990). Both clones reacted with only post inoculation antisera from infected cynos. The latter experiment confirmed that seroconversion in experimentally infected cynos was related to the isolated exogenous cloned sequence.

A composite cDNA sequence (obtained from several clones of the Mexican strain) is set forth below. Composite Mexico strain sequence (SEQ ID NO.10):

SEQ ID NO. 10:

	GCCATGGAGG CCCACCAGTT CATTAAAGCT CCTGGCATCA CTA CTGCTAT TGAGCAAGCA	60
25	GCTCTAGCAG CGGCCAACTC CGCCCTTGGC AATGCTGTGG TGGTCCGGCC TTTCCTTTCC	120
	CATCAGCAGG TTGAGATCCT TATAAATCTC ATGCAACCTC GGCAGCTGGT GTTTCGTCCT	180
	GAGGTTTTTT GGAATCACCC GATTCAACGT GTTATACATA ATGAGCTTGA GCAGTATTGC	240
30	CGTGCTCGCT CGGGTCGCTG CCTTGAGATT GGAGCCCACC CACGCTCCAT TAATGATAAT	300
	CCTAATGTCC TCCATCGCTG CTTTCTCCAC CCCGTCGGCC GGGATGTTCA GCGCTGGTAC	360
35	ACAGCCCCGA CTAGGGGACC TGC GGCGAAC TGTCGCCGCT CGGCACTTCG TGGTCTGCCA	420
	CCAGCCGACC GCACTTACTG TTTTGATGGC TTTGCCGGCT GCCGTTTTCG CGCCGAGACT	480
	GGTGTGGCTC TCTATTCTCT CCATGACTTG CAGCCGGCTG ATGTTGCCGA GCGATGGCT	540
40	CGCCACGGCA TGACCCGCCT TTATGCAGCT TTCCACTTGC CTCCAGAGGT GCTCCTGCCT	600
	CCTGGCACCT ACCGGACATC ATCCTACTTG CTGATCCACG ATGGTAAGCG CGCGGTTGTC	660
45	ACTTATGAGG GTGACACTAG CGCCGGTTAC AATCATGATG TTGCCACCCT CCGCACATGG	720

	ATCAGGACAA CTAAGGTTGT GGGTGAACAC CCTTTGGTGA TCGAGCGGGT GCGGGGTATT	780
	GGCTGTCACT TTGTGTTGTT GATCACTGCG GCCCCTGAGC CCTCCCCGAT GCCCTACGTT	840
5	CCTTACCCGC GTTCGACGGA GGTCTATGTC CGGTCTATCT TTGGGCCCGG CGGGTCCCCG	900
	TCGCTGTTCC CGACCGCTTG TGCTGTCAAG TCCACTTTTC ACGCCGTCCC CACGCACATC	960
	TGGGACCGTC TCATGCTCTT TGGGGCCACC CTCGACGACC AGGCCTTTTG CTGCTCCAGG	1020
10	CTTATGACGT ACCTTCGTGG CATTAGCTAT AAGGTAAGT TGGGTGCCCT GGTGCTAAT	1080
	GAAGGCTGGA ATGCCACCGA GGATGCGCTC ACTGCAGTTA TTACGGCGGC TTACCTCACA	1140
15	ATATGTCATC AGCGTTATTT GCGGACCCAG GCGATTTCTA AGGGCATGCG CCGGCTTGAG	1200
	CTTGAACATG CTCAGAAATT TATTTACGCG CTCTACAGCT GGCTATTTGA GAAGTCAGGT	1260
	CGTGATTACA TCCAGGCCG CAGCTGCAG TTCTACGCTC AGTGCCGCCG CTGGTTATCT	1320
20	GCCGGGTTCC ATCTCGACCC CCGCACCTTA GTTTTGTATG AGTCAGTGCC TTGTAGCTGC	1380
	CGAACCACCA TCCGGCGGAT CGCTGGAAAA TTTTGCTGTT TTATGAAGTG GCTCGGTCAG	1440
25	GAGTGTCTTT GTTTCCTCCA GCCCGCCGAG GGGCTGGCGG GCGACCAAGG TCATGACAA	1500
	GAGGCCTATG AAGGCTCTGA TGTGATACT GCTGAGCCTG CCACCCTAGA CATTACAGGC	1560
	TCATACATCG TGGATGGTCG GTCTCTGCAA ACTGTCTATC AAGCTCTCGA CCTGCCAGCT	1620
30	GACCTGGTAG CTCGCGCAGC CCGACTGTCT GCTACAGTTA CTGTTACTGA AACCTCTGGC	1680
	CGTCTGGATT GCCAAACAAT GATCGGCAAT AAGACTTTTC TCACTACCTT TGTTGATGGG	1740
35	GCACGCCTTG AGGTTAACGG GCCTGAGCAG CTTAACCTCT CTTTGTACAG CCAGCAGTGT	1800
	AGTATGGCAG CCGGCCCGTT TTGCCTCACC TATGCTGCCG TAGATGGCGG GCTGGAAGTT	1860
	CATTTTTCCA CCGTGGCCT CGAGAGCCGT GTTGTCTTCC CCCCTGGTAA TGCCCCGACT	1920
40	GCCCCGCCGA GTGAGGTAC CGCCTTCTGC TCAGCTCTTT ATAGGCACAA CCGGCAGAGC	1980
	CAGCGCCAGT CGGTTATTGG TAGTTTGTGG CTGACCCTG AAGGTTTGCT CGGCCTGTTC	2040
45	CCGCCCTTTT CACCCGGGCA TGAGTGCGG TCTGCTAACC CATTTTGCAG CGAGAGCACG	2100
	CTCTACACCC GCACTTGGTC CACAATTACA GACACACCCT TAACTGTGCG GCTAATTTCC	2160
	GGTCATTTGG ATGCTGCTCC CCACTCGGGG GGGCCACCTG CTAAGTCCAC AGGCCTGCT	2220
50	GTAGGCTCGT CTGACTCTCC AGACCCTGAC CCGCTACCTG ATGTTACAGA TGGCTCACGC	2280
	CCCTCTGGGG CCCGTCCGGC TGGCCCCAAC CCGAATGGCG TTCCGCAGCG CCGCTTACTA	2340
55	CACACCTACC CTGACGGCGC TAAGATCTAT GTCGGCTCCA TTTTCGAGTC TGAGTGCACC	2400

	TGGCTTGTC ACGCATCTAA CGCCGGCCAC CGCCCTGGTG GCGGGCTTTG TCATGCTTTT	2460
	TTTCAGCGTT ACCCTGATTC GTTTGACGCC ACCAAGTTTG TGATGCGTGA TGGTCTTGCC	2520
5	GCGTATACCC TTACACCCCG GCCGATCATT CATGCGGTGG CCCCAGACTA TCGATTGGAA	2580
	CATAACCCCA AGAGGCTCGA GGCTGCCTAC CGCGAGACTT GCGCCCGCCG AGGCACTGCT	2640
	GCCTATCCAC TCTTAGGCGC TGGCATTAC CAGGTGCCTG TTAGTTTGAG TTTTGATGCC	2700
10	TGGGAGCGGA ACCACCGCCC GTTTGACGAG CTTTACCTAA CAGAGCTGGC GGCTCGGTGG	2760
	TTTGAATCCA ACCGCCCCGG TCAGCCCACG TTGAACATAA CTGAGGATAC CGCCCGTGCG	2820
15	GCCAACCTGG CCCTGGAGCT TGACTCCGGG AGTGAAGTAG GCCGCGCATG TGCCGGGTGT	2880
	AAAGTCGAGC CTGGCGTTGT GCGGTATCAG TTTACAGCCG GTGTCCCGG CTCTGGCAAG	2940
	TCAAAGTCGG TGCAACAGGC GGATGTGGAT GTTGTGTTG TGCCCACTCG CGAGCTTCGG	3000
20	AACGCTTGGC GGCGCCGGGG CTTTGC GGCA TTTACTCCGC AACTGCGGC CCGTGTCACT	3060
	AGCGGCCGTA GGGTTGTCAT TGATGAGGCC CCTTCGCTCC CCCCACACTT GCTGCTTTTA	3120
25	CATATGCAGC GTGCTGCATC TGTGCACCTC CTTGGGGACC CGAATCAGAT CCCC GCCATA	3180
	GATTTTGAGC ACACCGGTCT GATTCCAGCA ATACGGCCGG AGTTGGTCCC GACTTCATGG	3240
	TGGCATGTCA CCCACCGTTG CCCTGCAGAT GTCTGTGAGT TAGTCCGTGG TGCTTACCCT	3300
30	AAAATCCAGA CTACAAGTAA GGTGCTCCGT TCCCTTTTCT GGGGAGAGCC AGCTGTGCGC	3360
	CAGAAGCTAG TGTTACACA GGCTGCTAAG GCCGCGCACC CCGGATCTAT AACGGTCCAT	3420
35	GAGGCCCAGG GTGCCACTTT TACCACTACA ACTATAATTG CAACTGCAGA TGCCCGTGCC	3480
	CTCATAAGT CCTCCCGGGC TCACGCTATA GTTGCTCTCA CTAGGCATAC TGAAAAATGT	3540
	GTTATACTTG ACTCTCCCGG CCTGTTGCGT GAGGTGGGTA TCTCAGATGC CATTGTTAAT	3600
40	AATTTCTTCC TTTCCGGTGG CGAGGTTGGT CACCAGAGAC CATCGGTCAT TCCGCGAGGC	3660
	AACCCTGACC GCAATGTTGA CGTGCTTGGC GCGTTTCCAC CTTCATGCCA AATAAGCGCC	3720
45	TTCCATCAGC TTGCTGAGGA GCTGGGCCAC CGGCCGGCGC CGGTGGCGGC TGTGCTACCT	3780
	CCCTGCCCTG AGCTTGAGCA GGGCCTTCTC TATCTGCCAC AGGAGCTAGC CTCCTGTGAC	3840
	AGTGTGTTGA CATTTGAGCT AACTGACATT GTGCACTGCC GCATGGCGGC CCCTAGCCAA	3900
50	AGGAAAGCTG TTTTGTCCAC GCTGGTAGGC CGGTATGGCA GACGCACAAG GCTTTATGAT	3960
	GCGGGTCACA CCGATGTCCG CGCCTCCCTT GCGCGCTTTA TTCCCACTCT CGGGCGGGTT	4020
55	ACTGCCACCA CCTGTGAAC TTTGAGCTT GTAGAGGCGA TGGTGGAGAA GGGCCAAGAC	4080

	GGTTCAGCCG TCCTCGAGTT GGATTTGTGC AGCCGAGATG TCTCCGCAT AACCTTTTTC	4140
	CAGAAGGATT GTAACAAGTT CACGACCGGC GAGACAATTG CGCATGGCAA AGTCGGTCAG	4200
5	GGTATCTCC GCTGGAGTAA GACGTTTTGT GCCCTGTTTG GCCCCTGGTT CCGTGCGATT	4260
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10	TCAGTATTCT CTGCTGCCGT GGCTGGCGCC AGCCATGCCA TGGTGTGGA AAATGATTTT	4380
	TCTGAGTTTG ACTCGACTCA GAATAACTTT TCCCTAGGTC TTGAGTGCGC CATTATGGAA	4440
	GAGTGTGGTA TGCCCCAGTG GCTTGTCAGG TTGTACCATG CCGTCCGGTC GGCCTGGATC	4500
15	CTGCAGGCCC CAAAAGAGTC TTTGAGAGGG TTCTGGAAGA AGCATTCTGG TGAGCCGGGC	4560
	AGCTTGCTCT GGAATACGGT GTGGAACATG GCAATCATTG CCCATTGCTA TGAGTTCCGG	4620
20	GACCTCCAGG TTGCCGCCCTT CAAGGGCGAC GACTCGGTCG TCCTCTGTAG TGAATACCGC	4680
	CAGAGCCCAG GCGCCGGTTC GCTTATAGCA GGCTGTGGTT TGAAGTTGAA GGCTGACTTC	4740
	CGGCCGATTG GGCTGTATGC CGGGGTGTC GTCGCCCCGG GGCTCGGGGC CCTACCCGAT	4800
25	GTCGTTCGAT TCGCCGGACG GCTTTCGGAG AAGAACTGGG GGCTGATCC GGAGCGGGCA	4860
	GAGCAGCTCC GCCTCGCCGT GCAGGATTTT CTCCGTAGGT TAACGAATGT GGCCAGATT	4920
30	TGTGTTGAGG TGGTGTCTAG AGTTTACGGG GTTCCCCGG GTCTGGTTCA TAACCTGATA	4980
	GGCATGCTCC AGACTATTGG TGATGGTAAG GCGCATTTTA CAGAGTCTGT TAAGCTATA	5040
	CTTGACCTTA CAACTCAAT TATGCACCGG TCTGAATGAA TAACATGTGG TTTGCTGCGC	5100
35	CCATGGGTTT GCCACCATGC GCCCTAGGCC TCTTTTGCTG TTGTTCTCT TGTTCCTGCC	5160
	TATGTTGCCC GCGCCACCGA CCGGTCAGCC GTCTGGCCGC CGTCGTGGGC GCGCAGCGG	5220
40	CGGTACCGGC GGTGGTTTCT GGGGTGACCG GGTGATTCT CAGCCCTTCG CAATCCCTTA	5280
	TATTCATCCA ACCAACCCTT TTGCCCCAGA CGTTGCCGCT GCGTCCGGGT CTGGACCTCG	5340
	CCTTCGCCAA CCAGCCCGGC CACTTGGCTC CACTTGCGGA GATCAGGCCC AGCGCCCTC	5400
45	CGCTGCCTCC CGTCGCCGAC CTGCCACAGC CGGGGCTGCG GCGCTGACGG CTGTGGCGCC	5460
	TGCCCATGAC ACCTCACCAG TCCCGGACGT TGATTCTCGC GGTGCAATC TACGCCGCA	5520
50	GTATAATTTG TCTACTTCAC CCCTGACATC CTCTGTGGCC TCTGGCACTA ATTAGTCTT	5580
	GTATGCAGCC CCCCTTAATC CGCCTCTGCC GCTGCAGGAC GGTACTAATA CTCACATTAT	5640
	GGCCACAGAG GCCTCCAATT ATGCACAGTA CCGGGTTGCC GCGGCTACTA TCCGTTACCG	5700
55	GCCCCTAGTG CCTAATGCAG TTGGAGGCTA TGCTATATCC ATTTCTTTCT GGCTCAAAC	5760

	AACCACAACC CCTACATCTG TTGACATGAA TTCCATTACT TCCACTGATG TCAGGATTCT	5820
	TGTTCAACCT GGCATAGCAT CTGAATTGGT CATCCCAAGC GAGCGCCTTC ACTACCGCAA	5880
5	TCAAGGTTGG CGCTCGGTTG AGACATCTGG TGTGCTGAG GAGGAAGCCA CCTCCGGTCT	5940
	TGTCATGTTA TGCATACATG GCTCTCCAGT TAACTCCTAT ACCAATACCC CTTATACCGG	6000
10	TGCCCTTGGC TTACTGGACT TTGCCTTAGA GCTTGAGTTT CGCAATCTCA CCACCTGTAA	6060
	CACCAATACA CGTGTGTCCC GTTACTCCAG CACTGCTCGT CACTCCGCCC GAGGGGCCGA	6120
	CGGGACTGCG GAGCTGACCA CAACTGCAGC CACCAGGTTT ATGAAAGATC TCCACTTTAC	6180
15	CGGCCTTAAT GGGGTAGGTG AAGTCGGCCG CGGGATAGCT CTAACATTAC TTAACCTTGC	6240
	TGACACGCTC CTCGGCGGGC TCCCGACAGA ATTAATTTTC TCGGCTGGCG GGCAACTGTT	6300
20	TTATTCCCGC CCGGTTGTCT CAGCCAATGG CGAGCCAACC GTGAAGCTCT ATACATCAGT	6360
	GGAGAATGCT CAGCAGGATA AGGGTGTTC TATCCCCAC GATATCGATC TTGGTGATTC	6420
	GCGTGTGGTC ATTCAGGATT ATGACAACCA GCATGAGCAG GATCGGCCCA CCCCCTCGCC	6480
25	TGCGCCATCT CGGCCTTTTT CTGTTCTCG AGCAAATGAT GTACTTTGGC TGTCCCTCAC	6540
	TGCAGCCGAG TATGACCAGT CCACTTACGG GTCGTCAACT GGCCCGGTTT ATATCTCGGA	6600
30	CAGCGTGACT TTGGTGAATG TTGCGACTGG CGCGCAGGCC GTAGCCCGAT CGCTTGACTG	6660
	GTCCAAAGTC ACCCTCGACG GCGGCCCCCT CCCGACTGTT GAGCAATATT CCAAGACATT	6720
	CTTTGTGCTC CCCCTTCGTG GCAAGCTCTC CTTTTGGGAG GCCGGCACAA CAAAAGCAGG	6780
35	TTATCCTTAT AATTATAATA CTACTGCTAG TGACCAGATT CTGATTGAAA ATGCTGCCGG	6840
	CCATCGGGTC GCCATTTCAA CCTATACCAC CAGGCTTGGG GCCGGTCCGG TCGCCATTTT	6900
40	TGCGGCCGCG GTTTTGGCTC CACGCTCCGC CCTGGCTCTG CTGGAGGATA CTTTTGATTA	6960
	TCCGGGGCGG GCGCACACAT TTGATGACTT CTGCCCTGAA TGCCGCGCTT TAGGCCTCCA	7020
	GGGTTGTGCT TTCCAGTCAA CTGTCGCTGA GCTCCAGCGC CTTAAAGTTA AGGTGGGTAA	7080
45	AACTCGGGAG TTGTAGTTTA TTTGGCTGTG CCCACCTACT TATATCTGCT GATTCCTTT	7140
	ATTCCTTTT TCTCGGTCCC GCGCTCCCTG A	7171

50 The above sequence was obtained from
polyadenylated clones. For clarity the 3' polyA
"tail" has been omitted.

The sequence above includes a partial cDNA sequence consisting of 1661 nucleotides that was identified in a previous application in this series. The previously identified partial sequence is set forth below, with certain corrections (SEQ ID NO.11). The corrections include deletion of the first 80 bases of the prior reported sequence, which are cloning artifacts; insertion of G after former position 174, of C after 270, and of GGCG after 279; change of C to T at former position 709, of GC to CG at 722-723, of CC to TT at 1238-39, and of C to G at 1606; deletion of T at former position 765; and deletion of the last 11 bases of the former sequence, which are part of a linker sequence and are not of viral origin.

15 Non-A Non-B T: Mexican Strain; SEQ ID NO.11
 SEQ ID NO. 11:

	GTTGCGTGAG GTGGGTATCT CAGATGCCAT TGTTAATAAT TTCTTCCTTT CGGGTGCGGA	60
20	GGTTGGTCAC CAGAGACCAT CGGTCATTCC GCGAGGCAAC CCTGACCGCA ATGTTGACGT	120
	GCTTGCGGCG TTTCCACCTT CATGCCAAAT AAGCGCCTTC CATCAGCTTG CTGAGGAGCT	180
	GGGCCACCGG CCGGCGCCGG TGGCGGCTGT GCTACCTCCC TGCCCTGAGC TTGAGCAGGG	240
25	CCTTCTCTAT CTGCCACAGG AGCTAGCCTC CTGTGACAGT GTTGTGACAT TTGAGCTAAC	300
	TGACATTGTG CACTGCCGCA TGCGGGCCCC TAGCCAAAGG AAAGCTGTTT TGCCACGCT	360
30	GGTAGGCCGG TATGGCAGAC GCACAAGGCT TTATGATGCG GGTACACCG ATGTCCGCGC	420
	CTCCCTTGCG CGCTTTATTC CCACTCTCGG GCGGGTTACT GCCACCACCT GTGAACCTTT	480
	TGAGCTTGTA GAGGCGATGG TGGAGAAGGG CCAAGACGGT TCAGCCGTCC TCGAGTTGGA	540
35	TTTGTGCAGC CGAGATGTCT CCCGCATAAC CTTTTTCCAG AAGGATTGTA ACAAGTTCAC	600
	GACCGGCGAG ACAATTGCGC ATGGCAAAGT CGGTCAGGGT ATCTTCGCT GGAGTAAGAC	660
40	CTTTTGTGCC CTGTTTGCC CCTGGTTCCG TGCGATTGAG AAGGCTATTC TATCCCTTTT	720
	ACCACAAGCT GTGTTCTACG GGGATGCTTA TGACGACTCA GTATTCTCTG CTGCCGTGGC	780
	TGGCGCCAGC CATGCCATGG TGTGAAAA TGATTTTCT GAGTTTGACT CGACTCAGAA	840
45	TAACTTTTCC CTAGGTCTTG AGTGCGCCAT TATGGAAGAG TGTGGTATGC CCCAGTGGCT	900
	TGTCAGGTTG TACCATGCCG TCCGGTCGGC GTGGATCCTG CAGGCCCAA AAGAGTCTTT	960

GAGAGGGTTC TGAAGAAGC ATTCTGGTGA GCCGGGCACG TTGCTCTGGA ATACGGTGTG 1020
 GAACATGGCA ATCATTGCCC ATTGCTATGA GTTCCGGGAC CTCCAGGTTG CCGCCTTCAA 1080
 5 GGGCGACGAC TCGGTCGTCC TCTGTASTGA ATACCGCCAG AGCCCAGGCG CCGGTTGCGT 1140
 TATAGCAGGC TGTGGTTTGA AGTTGAAGGC TGACTTCCGG CCGATTGGGC TGTATGCCGG 1200
 GGTGTGCTC GCCCGGGGC TCGGGGCCCT ACCCGATGTC GTTCGATTG CCGGACGGCT 1260
 10 TTCGGAGAAG AACTGGGGGC CTGATCCGGA GCGGGCAGAG CAGCTCCGCC TCGCCGTGCA 1320
 GGATTTCTC CGTAGGTTAA CGAATGTGGC CCAGATTGT GTTGAGGTGG TGTCTAGAGT 1380
 15 TTACGGGGTT TCCCCGGGTC TGGTTCATAA CCTGATAGGC ATGCTCCAGA CTATTGGTGA 1440
 TGGTAAGGCG CATTTTACAG AGTCTGTAA GCCTATACTT GACCTTACAC ACTCAATTAT 1500
 GCACCGGTCT GAATGAATAA CATGTGGTTT GCTGCGCCCA TGGGTTGCC ACCATGCGCC 1560
 20 CTAGGCCTCT TTTGC 1575

25 When comparing the Burmese and Mexican strains, 75.7% identity is seen in a 7189 nucleotide overlap beginning at nucleotide 1 of the Mexican strain and nucleotide 25 of the Burmese strain.

30 In the same manner, a different strain of HEV was identified in an isolate obtained in Tashkent, U.S.S.R. The Tashkent sequence is given below (SEQ ID NO.12):

SEQ ID NO. 12:

35 CGGGCCCCGT ACAGGTCACA ACCTGTGAGT TGTACGAGCT AGTGGAGGCC ATGGTCGAGA 60
 AAGGCCAGGA TGGCTCCGCC GTCCTTGAGC TCGATCTCTG CAACCGTGAC GTGTCCAGGA 120
 TCACCTTTT CCAGAAAGAT TGCAATAAGT TCACCACGGG AGAGACCATC GCCCATGGTA 180
 40 AAGTGGGCCA GGGCATTTCG GCCTGGAGTA AGACCTTCTG TGCCCTTTTC GGCCCTGGT 240
 TCCGTGCTAT TGAGAAGGCT ATTCTGGCCC TGCTCCCTCA GGGTGTGTTT TATGGGGATG 300
 CCTTTGATGA CACCGTCTTC TCGGCGCGTG TGGCCGAGC AAAGGCGTCC ATGGTGTGTTG 360
 45 AGAATGACTT TTCTGAGTTT GACTCCACCC AGAATAATTT TTCCCTGGGC CTAGAGTGTG 420
 CTATTATGGA GAAGTGTGGG ATGCCGAAGT GGCTCATCCG CTTGTACCAC CTTATAAGGT 480
 50 CTGCGTGGAT CCTGCAGGCC CCGAAGGAGT CCCTGCGAGG GTGTTGGAAG AAACACTCCG 540
 GTGAGCCCGG CACTCTTCTA TGAATACTG TCTGGAACAT GGCCGTTATC ACCCATTGTT 600

ACGATTTCCG CGATTTGCAG GTGGCTGCCT TTAAAGGTGA TGATTCGATA GTGCTTTGCA 660
 GTGAGTACCG TCAGAGTCCA GGGGCTGCTG TCCTGATTGC TGGCTGTGGC TTAAAGCTGA 720
 5 AGGTGGGTTT CCGTCCGATT GGTGTGTATG CAGGTGTTGT GGTGACCCCC GGCCTTGCCG 780
 CGCTTCCCGA CGTCGTGCGC TTGTCCGGCC GGCTTACTGA GAAGAATTGG GGCCCTGGCC 840
 10 CTGAGCGGGC GGAGCAGCTC CGCCTTGCTG TGCG 874

As shown in the following comparison of sequences, the Tashkent (Tash.) sequence more closely resembles the Burma sequence than the Mexico sequence, as would be expected of two strains from more closely related geographical areas. The numbering system used in the comparison is based on the Burma sequence. As indicated previously, Burma has SEQ ID NO:6; Mexico, SEQ ID NO:10; and Tashkent, SEQ ID NO:12. The letters present in the lines between the sequences indicate conserved nucleotides.

		10v	20v	30v	40v	50v	60v
25	-BURMA	AGGCAGACCACATATGTGGTCGATGCCATGGAGGCCCATCAGTTTATTAAGGCTCCTGGCA					
				GCCATGGAGGCCCA	CAGTT	ATTAAGGCTCCTGGCA	
	-MEXICO			GCCATGGAGGCCCA	CAGTT	ATTAAGGCTCCTGGCA	
		70v	80v	90v	100v	110v	120v
30	-BURMA	TCATACTGCTATTGAGCAGGCTGCTCTAGCAGCGGCCAACTCTGCCCTGGCGAATGCTG					
		TCATACTGCTATTGAGCA	GC	GCTCTAGCAGCGGCCAACTC	GCCCT	GCGAATGCTG	
	-MEXICO	TCATACTGCTATTGAGCAAGCAGCTCTAGCAGCGGCCAACTCCGCCCTTGCGAATGCTG					
		130v	140v	150v	160v	170v	180v
35	-BURMA	TGGTAGTTAGGCCTTTTCTCTCTCACCAGCAGATTGAGATCCTTAACCTAATGCAAC					
		TGGT GT	GGCCTTT CT TC	CA CAGCAG	TTGAGATCCT AT AA	CT ATGCAAC	
	-MEXICO	TGGTGGTCCGGCCTTTCTCTTCCCATCAGCAGGTTGAGATCCTTATAAATCTCATGCAAC					
		190v	200v	210v	220v	230v	240v
40	-BURMA	CTCGCCAGCTTGTTTTCCGCCCGAGGTTTTCTGGAATCATCCATCCAGCGTGTATCC					
		CTCG CAGCT GT TT CG CC	GAGGTTTT	TGGAATCA CC AT CA	CGTGT AT C		
	-MEXICO	CTCGGCAGCTGGTGTTCGTCTGAGGTTTTTGGAAATCACCCGATTCAACGTGTATAC					
		250v	260v	270v	280v	290v	300v
45	-BURMA	ATAACGAGCTGGAGCTTTACTGCCGCGCCGCTCCGGCCGCTGTCTTGAAATTGGCGCCC					
		ATAA GAGCT GAGC TA	TGCCG GC CGCTC GG	CGCTG CTTGA	ATTGG GCCC		
	-MEXICO	ATAATGAGCTTGAGCAGTATTGCCGTGCTCGCTCGGGTCGCTGCCTTGAGATTGGAGCCC					
		310v	320v	330v	340v	350v	360v
50	-BURMA	ATCCCCGCTCAATAAATGATAATCCTAATGTGGTCCACCGCTGCTTCTCCGCCCTGTTG					
		A CC CGCTC AT AATGATAATCCTAATGT	TCCA	CGCTGCTT CTCC	CCC GT G		
	-MEXICO	ACCCACGCTCCATTAATGATAATCCTAATGTCTCCATCGCTGCTTCTCCACCCGCTG					

		370v	380v	390v	400v	410v	420v
	-BURMA	GGCGTGATGTTTCAGCGCTGGTATCTGCTCCCACTCGCGGGCCGGCTGCTAATTGCCGGC					
5	-MEXICO	G CG GATGTTTCAGCGCTGGTA AC GC CC ACT G GG CC GC GC AA TG CG C					
		430v	440v	450v	460v	470v	480v
	-BURMA	GTTCCGCGCTGCGCGGACTTCCCGCTGCTGACGCACTTACTGCCTCGACGGGTTTTCTG					
10	-MEXICO	G TC GC CT CG GG CT CC C GC GACCGCACTTACTG T GA GG TTT C G					
		490v	500v	510v	520v	530v	540v
	-BURMA	GCTGTAACTTTCCCGCGAGACTGGCATCGCCCTCTACTCCCTTCATGATATGTACCAT					
15	-MEXICO	GCTG TTT CCGCGAGACTGG T GC CTCTA TC CT CATGA TG CC					
		550v	560v	570v	580v	590v	600v
	-BURMA	CTGATGTCGCCGAGGCCATGTTCCGCCATGGTATGACGCGGCTCTATGCCGCCCTCCATC					
20	-MEXICO	CTGATGT GCCGAGGC ATG CGCCA GG ATGAC CG CT TATGC GC TCCA					
		610v	620v	630v	640v	650v	660v
	-BURMA	TTCCGCGTGAGGTCTCTGCTGCCCTTGGCAGCATATCGACCGCATCGTATTTGCTAATTC					
25	-MEXICO	T CC CC GAGGT CT CTGCC CCTGGCAC TA CG AC CATC TA TTGCT AT C					
		670v	680v	690v	700v	710v	720v
	-BURMA	ATGACGGTAGGCGCGTTGTGGTGACGTATGAGGGTGATACTAGTGCTGGTTACAACCAG					
30	-MEXICO	A GA GGTA GCGCG GT GT AC TATGAGGGTGA ACTAG GC GGTACAA CA G					
		730v	740v	750v	760v	770v	780v
	-BURMA	ATGTCTCCAACCTTGCGCTCTGGATTAGAACCACCAAGGTTACCGGAGACCATCCCCTCG					
35	-MEXICO	ATGT CCA C T CGC C TGGAT AG AC AC AAGGTT GG GA CA CC T G					
		790v	800v	810v	820v	830v	840v
	-BURMA	TTATCGAGCGGGTTAGGGCCATTGGCTGCCACTTTGTTCTTGTCTCACGGCAGCCCCGG					
40	-MEXICO	T ATCGAGCGGGT GGG ATTGGCTG CACTTTGT T TTG TCAC GC GCCCC G					
		850v	860v	870v	880v	890v	900v
	-BURMA	AGCCATCACCTATGCCTTATGTTCTTACCCCGGTCTACCGAGGTCTATGTCCGATCGA					
45	-MEXICO	AGCC TC CC ATGCC TA GTTCTTACCC CG TC AC GAGGTCTATGTCCG TC A					
		910v	920v	930v	940v	950v	960v
	-BURMA	TCTTCGGCCCCGGGTGGCACCCCTTCTTATTCCCAACCTCATGCTCCACTAAGTCGACCT					
50	-MEXICO	TCTT GG CC GG GG CCCC TC T TTCC ACC C TG C AAGTC AC T					
		970v	980v	990v	1000v	1010v	1020v
	-BURMA	TCCATGCTATCCCTGCCCATATTTGGGACCGTCTTATGCTGTTCCGGGGCCACCTTGGATG					
55	-MEXICO	T CA GC GTCCC C CA AT TGGGACCGTCT ATGCT TT GGGGCCACC T GA G					

		1030v	1040v	1050v	1060v	1070v	1080v
	-BURMA	ACCAAGCCTTTTGTGCTCCCGTTTAAATGACCTACCTTCGCGGCATTAGCTACAAGGTCA					
5	-MEXICO	ACCA GCCTTTTGTGCTCC G T ATGAC TACCTTCG GGCATTAGCTA AAGGT A					
		1090v	1100v	1110v	1120v	1130v	1140v
	-BURMA	CTGTTGGTACCCTTGTGGCTAATGAAGGCTGGAATGCCTCTGAGGACGCCCTCACAGCTG					
10	-MEXICO	CTGT GGT CCCT GT GCTAATGAAGGCTGGAATGCC C GAGGA GC CTCAC GC G					
		1150v	1160v	1170v	1180v	1190v	1200v
	-BURMA	TTATCACTGCCGCTACCTTACCATTGCCACCAGCGGTATCTCCGACCCAGGCTATAT					
15	-MEXICO	TTAT AC GC GC TACCT AC AT TG CA CAGCG TAT T CG ACCCAGGC AT T					
		1210v	1220v	1230v	1240v	1250v	1260v
	-BURMA	CCAAGGGGATGCGTCTGGAACGGGAGCATGCCAGAAAGTTTATAACACGCCTCTACA					
20	-MEXICO	C AAGGG ATGCG CG CT GA C GA CATGC CAGAA TTTAT CACGCCTCTACA					
		1270v	1280v	1290v	1300v	1310v	1320v
	-BURMA	GCTGGCTCTTCGAGAAGTCCGGCCGTGATTACATCCCTGGCCGTGAGTTGGAGTTCTACG					
25	-MEXICO	GCTGGCT TT GAGAAGTC GG CGTGATTACATCCC GGCCG CAG TG AGTTCTACG					
		1330v	1340v	1350v	1360v	1370v	1380v
	-BURMA	CCCAGTGCAGGCGCTGGCTCTCCGCGGCTTTCATCTTGATCCAGGGTGTTGGTTTTTG					
30	-MEXICO	C CAGTGC G CGCTGG T TC GCCGG TT CATCT GA CC CG TT GTTTTTG					
		1390v	1400v	1410v	1420v	1430v	1440v
	-BURMA	ACGAGTCGGCCCCCTGCCATTGTAGGACCGCATCCGTAAGGCGCTCTCAAAGTTTTGCT					
35	-MEXICO	A GAGTC G CC TG TG G ACC C ATCCG G AAA TTTTGCT					
		1450v	1460v	1470v	1480v	1490v	1500v
	-BURMA	GCTTCATGAAGTGGCTTGGTCAGGAGTGCACCTGCTTCCTTCAGCCTGCAGAGGGCGCGG					
40	-MEXICO	G TT ATGAAGTGGCT GGTGAGGAGTG C TG TTCCT CAGCC GC GA GG G					
		1510v	1520v	1530v	1540v	1550v	1560v
	-BURMA	TCGGCGACCAAGGTCATGATAATGAAGCCTATGAGGGGTCCGATGTTGACCCTGCTGAGT					
45	-MEXICO	GGCGACCA GGTGATGA AATGA GCCTATGA GG TC GATGTTGA CTGCTGAG					
		1570v	1580v	1590v	1600v	1610v	1620v
	-BURMA	CCGCCATTAGTGACATATCTGGGTCCTATGTCGTCCTGGCACTGCCCTCAAACCGCTCT					
50	-MEXICO	C GCCA GACAT C GG TC TA TCGT TGG C CT CAA C TCT					
		1630v	1640v	1650v	1660v	1670v	1680v
	-BURMA	ACCAGGCCCTCGATCTCCCGCTGAGATTGTGGCTCGCGCGGGCGGCTGACCGCCACAG					
55	-MEXICO	A CA GC CTCGA CT CC GCTGA T GT GCTCGCGC G CCG CTG C GC ACAG					
		ATCAAGCTCTCGACCTGCCAGCTGACCTGGTAGCTCGCGCAGCCGACTGTCTGCTACAG					

		1690v	1700v	1710v	1720v	1730v	1740v
	-BURMA	TAAAGGTCTCCCAGGTCGATGGGCGGATCGATTGCGAGACCTTCTTGGTAACAAAACCT					
5	-MEXICO	T A GT C A C TGG CG T GATTGC A AC T T GG AA AA AC T					
		1750v	1760v	1770v	1780v	1790v	1800v
	-BURMA	TTCGCACGTCGTTGCTTGACGGGGCGGTCTTAGAGACCAATGGCCCAGAGCGCCACAATC					
10	-MEXICO	TTC CAC C TT GTTGA GGGGC C T GAG AA GG CC GAGC C AA C					
		1810v	1820v	1830v	1840v	1850v	1860v
	-BURMA	TCTCCTTCGATGCCAGTCAGAGCACTATGGCCGCTGGCCCTTTCAGTCTCACCTATGCCG					
15	-MEXICO	TCTC TT GA C CAG G A TATGGC GC GGCC TT G CTCACCTATGC G					
		1870v	1880v	1890v	1900v	1910v	1920v
	-BURMA	CCTCTGCAGCTGGGCTGGAGGTGCGCTATGTTGCTGCCGGGCTTGACCATCGGGCGGTTT					
20	-MEXICO	CC G G GGGCTGGA GT C T T C GC GG CT GA CG G GTTT					
		1930v	1940v	1950v	1960v	1970v	1980v
	-BURMA	TTGCCCCCGGTGTTTACCCCGGTGAGCCCCGCGAGGTTACCGCCTTCTGCTCTGCC					
25	-MEXICO	T CCCC GGT T C CC C C CC G GAGGT ACCGCCTTCTGCTC GC C					
		1990v	2000v	2010v	2020v	2030v	2040v
	-BURMA	TATACAGGTTTAACCGTGAGGCCAGCGCCATTGCTGATCGGTAACCTATGGTTCCATC					
30	-MEXICO	T TA AGG AACCG AG CCAGCGCCA TCG T AT GGTA TT TGG T CA C					
		2050v	2060v	2070v	2080v	2090v	2100v
	-BURMA	CTGAGGGACTCATTGGCCTCTTCGCCCGTTCGCCCCGGCATGTTTGGGAGTCGGCTA					
35	-MEXICO	CTGA GG T T GGCCT TTC C CC TTTTC CCCGGGCATG TGG GTC GCTA					
		2110v	2120v	2130v	2140v	2150v	2160v
	-BURMA	ATCCATTCTGTGGCGAGAGCACACTTTACACCCGACTTGGTCGGAGGTTGATGCCGTCT					
40	-MEXICO	A CCATT TG GGCAGAGCAC CT TACACCCG ACTTGGTC TT G C					
		2170v	2180v	2190v	2200v	2210v	2220v
	-BURMA	CTAGTCCAGCCCGCCTGACTTAGGTTTTATGTCTGAGCCTTCTATACCTAGTAGGGCCG					
45	-MEXICO	C C G C GGC T GGT T TG TG CT C C G GG C					
		2230v	2240v	2250v	2260v	2270v	2280v
	-BURMA	CCACGCCTACCCTGGCGGCCCTCTACCCCCCTGCACCGGACCCTTCCCCCTCCCT					
50	-MEXICO	C C CT CC G C CT TA C C CTG C C CCC C					
		2290v	2300v	2310v	2320v	2330v	2340v
	-BURMA	CTGCCCCGCGCTTGCTGAGCCGGCTTCTGGCGCTACCGCCGGGGCCCGGCATAACTC					
55	-MEXICO	CTG C TG C C TCTGG GC C G G CCC C A T					

		2350v	2360v	2370v	2380v	2390v	2400v
	-BURMA	ACCAGACGGCCCGGCACCGCCGCTGCTCTTACCTACCCGGATGGCTCTAAGGTATTG					
		C CG	CGCCGC T CT	CACCTACCC GA	GGC CTAAG T T	G	
5	-MEXICO	GCGTTCCGC4G-----CGCCGCTTACTACACCTACCCTGACGGCGCTAAGATCTATG					
		2410v	2420v	2430v	2440v	2450v	2460v
	-BURMA	CCGGCTCGCTGTTGAGTCGACATGCACGTGGCTCGTTAACGCGTCTAATGTTGACCACC					
		CGGCTC T TTCGAGTC	TGCAC TGGCT GT	AACGC TCTAA G	G CCACC		
10	-MEXICO	TGGCTCCATTTTCGAGTCTGAGTGCACCTGGCTTGCAACGCATCTAACGCCGGCCACC					
		2470v	2480v	2490v	2500v	2510v	2520v
	-BURMA	GCCCTGGCGGGGCTTTGCCATGCATTTTACCAAAGGTACCCCGCTCTTTGATGCTG					
		GCCCTGG GCGGGCTTTG	CATGC TTTT CA	G TACCC G	TC TTTGA GC		
15	-MEXICO	GCCCTGGTGGCGGGCTTTGTATGCTTTTTTTTTCAGCGTTACCCTGATTGTTTACGCCA					
		2530v	2540v	2550v	2560v	2570v	2580v
	-BURMA	CCTCTTTTGTGATGCGCGACGGCGCGGCCGTACACACTAACCCCCGGCCAATAATTC					
		CC TTTGTGATGCG GA GG	GCCGCGTA AC	CT AC CCCC	GGCC AT ATTC		
20	-MEXICO	CCAAGTTTGTGATGCGTGTGCTTGTCCGCGTATACCCTTACACCCGGCCGATCATTC					
		2590v	2600v	2610v	2620v	2630v	2640v
	-BURMA	ACGCTGTGCGCCCTGATTATAGGTTGGAACATAACCCAAAGAGGCTTGAGGCTGCTTATC					
		A GC GT GCCCC GA	TAT G TGGAAACATAACCC	AAGAGGCT	GAGGCTGC	TA C	
25	-MEXICO	ATGCGGTGGCCCCGGACTATCGATTGGAACATAACCCAAAGAGGCTCGAGGCTGCCTACC					
		2650v	2660v	2670v	2680v	2690v	2700v
	-BURMA	GGGAACTTGCTCCCGCTCGGCACCGCTGCATACCCGCTCCTCGGGACCGGCATATACC					
		G GA ACTTGC CCCGCC	GGCAC GCTGC	TA CC CTC	T GG C GGCAT	TACC	
30	-MEXICO	GCGAGACTTGCGCCCCGCCGAGGCACTGCTGCCTATCCACTCTTAGCGCTGGCATTTACC					
		2710v	2720v	2730v	2740v	2750v	2760v
	-BURMA	AGGTGCCGATCGGCCAGTTTTGACGCTGGGAGCGGAACACCAGCCCGGGGATGAGT					
		AGGTGCC T G	AGTTTTGA	GCCTGGGAGCGGAACACCAGCC	CCCC GA	GAG	
35	-MEXICO	AGGTGCCCTGTTAGTTTGTGTTTTGATGCTGGGAGCGGAACACCAGCCCGTTTGACGAGC					
		2770v	2780v	2790v	2800v	2810v	2820v
	-BURMA	TGTACCTTCTGAGCTTGCTGCCAGATGTTTGAGGCCAATAGGCCGACCCGCCGACTC					
		T TACCT C	GAGCT GC GC	G TGGTTTGA	CCAA G CC	C CC AC	
40	-MEXICO	TTTACCTAACAGAGCTGGCGGCTCGGTGTTTTGAATCCAACCGCCCGGTGAGCCACGT					
		2830v	2840v	2850v	2860v	2870v	2880v
	-BURMA	TCACTATAACTGAGGATGTTGCACGGAACAGCGA/	GTGGCCATCGAGCTTGACTCAGCCA				
		T A ATA	ACTGAGGAT GC CG	C GC AA	CTGGCC T	GAGCTTGACTC	G A
45	-MEXICO	TGAACATAACTGAGGATACCGCCCGTGGCGCCAACTGGCCCTGGAGCTTGACTCCGGGA					
		2890v	2900v	2910v	2920v	2930v	2940v
	-BURMA	CAGATGTGCGCCGGGCTGTGCCGGCTGTGCGGTACCCCCGGCGTTGTTTCACTACAGT					
		GA GT GGCCG GC	TGTGCCGG TGT	GTC CC	GCGTTGT C	GTA CAGT	
50	-MEXICO	GTGAAGTAGGCCGCGCATGTGCCGGGTGTAAAGTCGAGCCTGGCGTTGTGCGGTATCAGT					
		2950v	2960v	2970v	2980v	2990v	3000v
	-BURMA	TTACTGCAGGTGTGCTGGATCCGGCAAGTCCCGCTCTATACCCCAAGCCGATGTGGACG					
		TTAC GC	GGTGT CC GG	TC GGCAAGTC	TC T	CA GC	GATGTGGA G
55	-MEXICO	TTACAGCCGGTGTCCCGGCTCTGGCAAGTCAAAGTCCGTGCAACAGGCGGATGTGGATG					

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	3010v	3020v	3030v	3040v	3050v	3060v
-BURMA	TTGTCGTGGTCCCGACGCGTGAGTTGCGTAATGCCTGGCGCCGTCGCGGCTTTGCTGCTT					
-MEXICO	TTGT GT GT CC AC CG GAG T CG AA GC TGGCG CG CG GGCTTTGC GC T					
5						
	3070v	3080v	3090v	3100v	3110v	3120v
-BURMA	TTACCCCGCATACTGCCGCCAGAGTCACCCAGGGGCGCCGGGTTGTCATTGATGAGGGCTC					
-MEXICO	T AC CCGCA ACTGC GCC G GTCAC GG CG GGGTTGTCATTGATGAGGC C					
10						
	3130v	3140v	3150v	3160v	3170v	3180v
-BURMA	CATCCCTCCCCCTCACCTGCTGCTGCTCCACATGCAGCGGGCCGCCACCGTCCACCTTC					
-MEXICO	C TC CTCCCCC CAC TGCTGCT T CA ATGCAGCG GC GC C GT CACCT C					
15						
	3190v	3200v	3210v	3220v	3230v	3240v
-BURMA	TTGGCGACCCGAACCGATCCAGCCATCGACTTTGAGCACGCTGGGCTCGTCCCCGCCA					
-MEXICO	TTGG GACCCGAA CAGATCCC GCCAT GA TTTGAGCAC C GG CT T CC GC A					
20						
	3250v	3260v	3270v	3280v	3290v	3300v
-BURMA	TCAGGCCCGACTTAGGCCCCACCTCCTGGTGGCATGTTACCCATCGCTGGCTGCGGATG					
-MEXICO	T GGCC GA TT G CCC AC TC TGGTGGCATGT ACCCA CG TG CCTGC GATG					
25						
	3310v	3320v	3330v	3340v	3350v	3360v
-BURMA	TATGCGAGCTCATCCGTGGTGCATACCCATGATCCAGACCTAGCCGGGTTCTCCGTT					
-MEXICO	T TG GAG T TCCGTGGTGC TACCC A ATCCAGAC AC AG GGT CTCCGTT					
30						
	3370v	3380v	3390v	3400v	3410v	3420v
-BURMA	CGTTGTTCTGGGGTGAGCCTGCCGTGCGGCAGAACTAGTGTTACCCAGGCGGCCAAGC					
-MEXICO	C T TTCTGGGG GAGCC GC GTCGG CAGAA CTAGTGTTAC CAGGC GC AAG					
35						
	3430v	3440v	3450v	3460v	3470v	3480v
-BURMA	CCGCCAACCCCGGCTCAGTGACGGTCCACGAGGCGCAGGGCGCTACCTACAGGAGACCA					
-MEXICO	CCGC ACCCCGG TC T ACGGTCCA GAGGC CAGGG GC AC T AC AC A					
40						
	3490v	3500v	3510v	3520v	3530v	3540v
-BURMA	CTATTATTGCCACAGCAGATGCCCGGGGCTTATTCAAGTCGTCCTCGGGCTCATGCCATTG					
-MEXICO	CTAT ATTGC AC GCAGATGCCG GGCCT AT CAGTC TC CGGGCTCA GC AT G					
45						
	3550v	3560v	3570v	3580v	3590v	3600v
-BURMA	TTGCTCTGACGCGCCACACTGAGAAGTGCATCATTTGACGCACAGGCTGCTTCGCG					
-MEXICO	TTGCTCT AC G CA ACTGA AA TG GT AT TTGAC C CC GGCCTG T CG G					
50						
	3610v	3620v	3630v	3640v	3650v	3660v
-BURMA	AGGTGGGCTCTCCGATGCAATCGTTAATAACTTTTCTCCTCGTGGTGGCGAAATTGGTC					
-MEXICO	AGGTGGG ATCTC GATGC AT GTTAATAA TT TTCCT C GGTGGCGA TTGGTC					
55						

		3670v	3680v	3690v	3700v	3710v	3720v
	-BURMA	ACCAGCGCCCATCAGTTATTCCTCGTGGCAACCTGACGCCAATGTTGACACCCTGGCTG					
		ACCAG G CCATC GT ATTCC CG GGCAACCTGAC CAATGTTGAC CT GC G					
5	-MEXICO	ACCAGAGACCATCGGTCAATTCGCGAGGCAACCTGACCGCAATGTTGACGTGCTTGCGG					
		3730v	3740v	3750v	3760v	3770v	3780v
	-BURMA	CCTTCCCGCGTCTTGCCAGATTAGTGCTTCCATCAGTTGGCTGAGGAGCTTGCCACA					
		C TT CC CC TC TGCCA AT AG GCCTTCCATCAG T GCTGAGGAGCT GGCCAC					
10	-MEXICO	CGTTTCCACCTTCATGCCAAATAAGCGCTTCCATCAGCTTGCTGAGGAGCTGGGCCACC					
		3790v	3800v	3810v	3820v	3830v	3840v
	-BURMA	GACCTGTCCCTGTTGCAGCTGTTCTACCACCTGCCCGAGCTCGAACAGGGCCTTCTCT					
		G CC G CC GT GC GCTGT CTACC CCCTGCCC GAGCT GA CAGGGCCTTCTCT					
15	-MEXICO	GGCCGCGCCGGTGGCGGCTGTGTACCTCCCTGCCCTGAGCTTGAGCAGGGCCTTCTCT					
		3850v	3860v	3870v	3880v	3890v	3900v
	-BURMA	ACCTGCCCAAGGAGCTCACCACCTGTGATAGTGTGTAACATTTGAATTAACAGACATTG					
		A CTGCC CAGGAGCT CC CCTGTGA AGTGT GT ACATTTGA TAAC GACATTG					
20	-MEXICO	ATCTGCCACAGGAGCTAGCCTCCTGTGACAGTGTGTGACATTTGAGCTAACTGACATTG					
		3910v	3920v	3930v	3940v	3950v	3960v
	-BURMA	TGCACTGCCGCATGGCCGCCCGAGCCAGCGCAAGGCCGTGCTGTCCACACTCGTGGGCC					
		TGCACTGCCGCATGGC GCCC AGCCA G AA GC GT TGTCCAC CT GT GGCC					
25	-MEXICO	TGCACTGCCGCATGGCGGCCCTAGCCAAAGGAAAGCTGTTTTGTCCACGCTGGTAGGCC					
		3970v	3980v	3990v	4000v	4010v	4020v
	-BURMA	GCTACGGCGGTGCGACAAAGCTCTACAATGCTTCCCACTCTGATGTTGCGGACTCTCTCG					
		G TA GGC G CGACAA GCT TA ATGC CAC C GATGT CGCG CTC CT G					
30	-MEXICO	GGTATGGCAGACGACAAAGGCTTTATGATGCGGGTCACACCGATGTCGCGCCTCCCTTG					
		4030v	4040v	4050v	4060v	4070v	4080v
	-TASHKENT	GGCCCCGTACAGGTACAACTGTGAGTTGTACGAGCTAG					
		GGCCCCGTACAGGT ACAAC TGTGA TTGTACGAGCTAG					
35	-BURMA	CCCGTTTTATCCCGGCCATTGGCCCCGTACAGGTTACAACCTTGTGAATTGTACGAGCTAG					
		C CG TTTAT CC C T GG C GT G AC AC TGTGAA T T GAGCT G					
	-MEXICO	CGCGCTTTATTCCCACTCTCGGGCGGGTTACTGCCACCACCTGTGAACCTTTGAGCTTG					
		4090v	4100v	4110v	4120v	4130v	4140v
40	-TASHKENT	TGGAGGCCATGGTCGAGAAAGGCCAGGATGGCTCCGCCGTCTTGAGCTCGATCTCTGCA					
		TGGAGGCCATGGTCGAGAA GGCCAGGATGGCTCCGCCGTCTTGAGCT GATCT TGCA					
	-BURMA	TGGAGGCCATGGTCGAGAAAGGCCAGGATGGCTCCGCCGTCTTGAGCTTGATCTTTGCA					
		T GAGGC ATGGT GAGAAGGGCCA GA GG TC GCCGTCTT GAG T GAT T TGCA					
	-MEXICO	TAGAGGCGATGGTGGAGAAGGGCCAAGACGGTTCAGCCGTCTCGAGTTGGATTGTGCA					
45		4150v	4160v	4170v	4180v	4190v	4200v
	-TASHKENT	ACCGTGACGTGTCCAGGATCACCTTTTTCCAGAAAGATTGCAATAAGTTCCACCACGGGAG					
		ACCGTGACGTGTCCAGGATCACCTT TTCCAGAAAGATTG AA AAGTTCCACCAC GG G					
	-BURMA	ACCGTGACGTGTCCAGGATCACCTTCTTCCAGAAAGATTGTAACAAGTTCCACCACAGGTG					
		CCG GA GT TCC G AT ACCTT TTCCAGAA GATTGTAACAAGTTCCAC AC GG G					
50	-MEXICO	GCCGAGATGTCTCCGCATAACCTTTTTCCAGAAAGATTGTAACAAGTTCCAGCCGGCG					

		4210v	4220v	4230v	4240v	4250v	4260v	
	-TASHKENT	AGACCATCGCCCATGGTAAAGTGGGCCAGGGCATTTCGGCCTGGAGTAAGACCTTCTGTG						
		AGACCAT GCCCATGGTAAAGTGGGCCAGGGCATTTCGGCCTGGAG AAGACCTTCTG G						
5	-BURMA	AGACCATGCCCCATGGTAAAGTGGGCCAGGGCATCTCGGCCTGGAGCAAGACCTTCTGCG						
		AGAC ATTGC CATGG AAAGT GG CAGGG ATCT CTGGAG AAGAC TT TG G						
	-MEXICO	AGACAATTGCGCATGGCAAAGTCGGTCAGGGTATCTTCGCTGGAGTAAGACGTTTTGTG						
			4270v	4280v	4290v	4300v	4310v	4320v
10	-TASHKENT	CCCTTTTCGGCCCCCTGGTTCCGTGCTATTGAGAAGGCTATTCTGGCCCTGCTCCCTCAGG						
		CCCT TT GGGCC TGGTTCCG GCTATTGAGAAGGCTATTCTGGCCCTGCTCCCTCAGG						
	-BURMA	CCCTCTTTGGCCCCCTGGTTCCGCGCTATTGAGAAGGCTATTCTGGCCCTGCTCCCTCAGG						
		CCCT TTTGGCCC TGGTTCCG GC ATTGAGAAGGCTATTCT CCCT T CC CA G						
	-MEXICO	CCCTGTTTGGCCCCCTGGTTCCGTGCGATTGAGAAGGCTATTCTATCCCTTTTACCACAAG						
			4330v	4340v	4350v	4360v	4370v	4380v
15	-TASHKENT	GTGTGTTTTATGGGGATGCCTTTTGATGACACCGTCTTCTCGGCGCGTGTGGCCGAGCAA						
		GTGTGTTTTA GG GATGCCTTTGATGACACCGTCTTCTCGGCG TGTGGCCGAGCAA						
	-BURMA	GTGTGTTTTACGGTGATGCCTTTTGATGACACCGTCTTCTCGGCGCGTGTGGCCGAGCAA						
		TGTGTT TACGG GATGC T TGA GAC C GT TTCTC GC GC GTGGC G GC A						
20	-MEXICO	CTGTGTTCTACGGGGATGCTTATGACGACTCAGTATTCTCTGCTGCCGTGGCTGGCGCCA						
			4390v	4400v	4410v	4420v	4430v	4440v
25	-TASHKENT	AGGCGTCCATGGTGTTTGAGAATGACTTTTCTGAGTTTGACTCCACCCAGAATAATTTTT						
		AGGC TCCATGGTGTTTGAGAATGACTTTTCTGAGTTTGACTCCACCCAGAATAA TTTT						
	-BURMA	AGGCATCCATGGTGTTTGAGAATGACTTTTCTGAGTTTGACTCCACCCAGAATAACTTTT						
		CCATGGTGTTTGA AATGA TTTTCTGAGTTTGACTC AC CAGAATAACTTTT						
	-MEXICO	GCCATGCCATGGTGTTTGAAAATGATTTTTCTGAGTTTGACTCGACTCAGAATAACTTTT						
			4450v	4460v	4470v	4480v	4490v	4500v
30	-TASHKENT	CCCTGGGCCTAGAGTGTGCTATTATGGAGAAGTGTGGGATGCCGAAGTGCTCATCCGCT						
		C CTGGG CTAGAGTGTGCTATTATGGAG AGTGTGGGATGCCG AGTGGCTCATCCGC						
	-BURMA	CTCTGGGTCTAGAGTGTGCTATTATGGAGGAGTGTGGGATGCCGAGTGCTCATCCGCC						
		C CT GGTCT GAGTG GC ATTATGGA GAGTGTG ATGCC CAGTGGCT TC G						
35	-MEXICO	CCCTAGGTCTTGAGTGCGCCATTATGGAAGAGTGTGGTATGCCCCAGTGGCTTGTCAGGT						
			4510v	4520v	4530v	4540v	4550v	4560v
40	-TASHKENT	TGTACCACCTTATAAGGTCTGCGTGGATCCTGCAGGCCCCGAAGGAGTCCCTGCGAGGGT						
		TGTA CACCTTATAAGGTCTGCGTGGATC TGCAGGCCCCGAAGGAGTC CTGCGAGGGT						
	-BURMA	TGTATCACCTTATAAGGTCTGCGTGGATCTTGCAGGCCCCGAAGGAGTCTCTGCGAGGGT						
		TGTA CA T GGTC GCGTGGATC TGCAGGCCCC AA GAGTCT TG GAGGGT						
	-MEXICO	TGTACCATGCCGTCCGGTCGCGTGGATCCTGCAGGCCCCAAAGAGTCTTTGAGAGGGT						
			4570v	4580v	4590v	4600v	4610v	4620v
45	-TASHKENT	GTTGGAAGAAACACTCCGGTGAGCCCGGCACTCTTCTATGGAATACTGTCTGGAACATGG						
		TTGGAAGAAACACTCCGGTGAGCCCGGCACTCTTCTATGGAATACTGTCTGGAA ATGG						
	-BURMA	TTTGGAAGAAACACTCCGGTGAGCCCGGCACTCTTCTATGGAATACTGTCTGGAATATGG						
		T TGGAGAA CA TC GGTGAGCC GGCA T CT TGGAAATAC GT TGGAA ATGG						
	-MEXICO	TCTGGAAGAAGCATTCTGGTGAGCCGGGAGCTTGCTCTGGAATACGGTGTGGAACATGG						
			4630v	4640v	4650v	4660v	4670v	4680v
50	-TASHKENT	CCGTTATACCCATTGTTACGATTTCCGCGATTTCAGGTGGCTGCCTTTAAAGGTGATG						
		CCGTTAT ACCCA TGTTA GA TTCCGCGATT AGGTGGCTGCCTTTAAAGGTGATG						
	-BURMA	CCGTTATTACCCACTGTTATGACTTCCGCGATTTCAGGTGGCTGCCTTTAAAGGTGATG						
		C T ATT CCCA TG TATGA TTCCG GA T CAGGT GC GCCTT AA GG GA G						
55	-MEXICO	CAATCATTGCCATTGCTATGAGTTCCGGGACCTCCAGGTGCGCGCTTCAAGGGCGACG						

	4690v	4700v	4710v	4720v	4730v	4740v	
5	-TASHKENT	ATTCGATAGTGCTTTGCAGTGAGTACCGTCAGAGTCCAGGGGCTGCTGTCTGATTGCTG ATTCGATAGTGCTTTGCAGTGAGTA CGTCAGAGTCCAGG GCTGCTGTCTGAT GC G					
	-BURMA	ATTCGATAGTGCTTTGCAGTGAGTATCGTCAGAGTCCAGGAGCTGCTGTCTGATCGCCG A TCG T GT CT TG AGTA TA CG CAGAG CCAGG GC G T CT AT GC G					
	-MEXICO	ACTCGGTCGTCTCTGTAGTGAATACCGCCAGAGCCAGGCGCCGGTTCGCTTATAGCAG					
10		4750v	4760v	4770v	4780v	4790v	4800v
	-TASHKENT	GCTGTGGCTTAAAGCTGAAGGTGGGTTTCCGTCGATTGGTTTGATGTCAGGTGTTGTGG GCTGTGGCTT AAG TGAAGGT G TTTCCG CCGAT GGTGTTGATGTCAGGTGTTGTGG					
	-BURMA	GCTGTGGCTTGAAGTTGAAGGTAGATTTCGCCCCGATCGGTTTGATGTCAGGTGTTGTGG GCTGTGG TTGAAGTTGAAGG GA TTTCCG CCGAT GG TGTATGC GG GTTGT G					
	-MEXICO	GCTGTGGTTTGAAGTTGAAGGCTGACTTCCGGCCGATTGGGCTGTATGCCGGGGTTGTCC					
15		4810v	4820v	4830v	4840v	4850v	4860v
	-TASHKENT	TGACCCCCGGCCTTGGCGCGCTTCCCGACGTCGTGCGCTTGTCCGGCCGGCTTACTGAGA TG CCCCCGGCCTTGGCGCGCTTCCCGA GT GTGCGCTTG CCGGCCGGCTTAC GAGA					
	-BURMA	TGGCCCCCGGCTTGGCGCGCTTCCCTGATGTTGTGCGCTTCGCCGGCCGGCTTACCGAGA T GCCCC GG CT GG GC CT CC GATGT GT CG TTCGCCGG CGGCTT C GAGA					
	-MEXICO	TCGCCCGGGGCTCGGGGCCCTACCCGATGTCGTTTCGATTGCGCGGACGGCTTTCGGAGA					
20		4870v	4880v	4890v	4900v	4910v	4920v
	-TASHKENT	AGAATTGGGGCCCTGGCCCTGAGCGGGCGGAGCAGCTCCGCCTTGCTGT AGAATTGGGGCCCTGGCCCTGAGCGGGCGGAGCAGCTCCGCCT GCTGT					
	-BURMA	AGAATTGGGGCCCTGGCCCTGAGCGGGCGGAGCAGCTCCGCCTGCTGTTAGTGATTTCC AGAA TGGGG CCG CC GAGCGGGC GAGCAGCTCCGCCTCGC GT GATTTCC					
	-MEXICO	AGAACTGGGGCCCTGATCGGAGCGGGCAGAGCAGCTCCGCCTCGCGTGACAGGATTTCC					
25		4930v	4940v	4950v	4960v	4970v	4980v
	-BURMA	TCCGCAAGCTCACGAATGTAGCTCAGATGTGTGTGGATGTTGTTTCCCGTGTATGGGG TCCG A G T ACGAATGT SC CAGAT TGTGT GA GT GT TC G GTTTA GGGG					
	-MEXICO	TCCGTAGGTTAACGAATGTGGCCAGATTTGTGTTGAGGTGGTGTCTAGAGTTTACGGGG					
30		4990v	5000v	5010v	5020v	5030v	5040v
	-BURMA	TTTCCCTCGGACTCGTTATAACCTGATTGGCATGCTACAGGCTGTTGCTGATGGCAAGG TTTCCCC GG CT GTTATAACCTGAT GGCATGCT CAG CT TTG TGATGG AAGG					
	-MEXICO	TTTCCCCGGGCTCGGTTATAACCTGATAGGCATGCTCCAGACTATTGGTGATGGTAAGG					
35		5050v	5060v	5070v	5080v	5090v	5100v
	-BURMA	CACATTTCACTGAGTCAGTAAAACAGTGCTCGACTTGACAAATTCAATCTTGTGTGGG C CATTT AC GAGTC GT AA CC T CT GAC T ACA A TCAAT TG CGG					
	-MEXICO	CGCATTTTACAGAGTCTGTTAAGCCTATACTTGACCTTACACACTCAATTATGCACCGGT					
40		5110v	5120v	5130v	5140v	5150v	5160v
	-BURMA	TGGAATGAATAACATGTCTTTTGTGCTGCGCCCATGGGTTGCGACCATGCGCCCTCGGCCT GAATGAATAACATGT TTTGCTGCGCCCATGGGTTGCG ACCATGCGCCCT GGCCT					
	-MEXICO	CTGAATGAATAACATGTGGTTTGTGCTGCGCCCATGGGTTGCCACCATGCGCCCTAGGCCT					
45		5170v	5180v	5190v	5200v	5210v	5220v
	-BURMA	ATTTTGTGTGCTGCTCCTCATGTTTTTGCCTATGCTGCCCGGCCACCGCCGGTCAGCCG TTTTG TG TG TCCTC TGTGT TGCTATG TGCCCGGCCACCG CCGGTCAGCCG					
	-MEXICO	CTTTTGTGTGTTTCTCTGTTTCTGCCTATGTTGCCCGGCCACCGACCGGTCAGCCG					
50		5170v	5180v	5190v	5200v	5210v	5220v
	-BURMA	ATTTTGTGTGCTGCTCCTCATGTTTTTGCCTATGCTGCCCGGCCACCGCCGGTCAGCCG TTTTG TG TG TCCTC TGTGT TGCTATG TGCCCGGCCACCG CCGGTCAGCCG					
	-MEXICO	CTTTTGTGTGTTTCTCTGTTTCTGCCTATGTTGCCCGGCCACCGACCGGTCAGCCG					

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		5230v	5240v	5250v	5260v	5270v	5280v
5	-BURMA	TCTGGCCGCGTCGTGGGCGGCGCAGCGCGGTTCGGCGGTGGTTTCTGGGGTGACCGG					
	-MEXICO	TCTGGCCGCGTCGTGGGCGGCGCAGCGCGGT CCGCGGTGGTTTCTGGGGTGACCGG					
		5290v	5300v	5310v	5320v	5330v	5340v
	-BURMA	GTTGATTCTCAGCCCTTCGCAATCCCCTATATTTCATCCAACCAACCCCTTCGCCCCGAT					
10	-MEXICO	GTTGATTCTCAGCCCTTCGCAATCCCCTATATTTCATCCAACCAACCCCTT GCCCC GA					
		5350v	5360v	5370v	5380v	5390v	5400v
	-BURMA	GTCACCGCTGCGGCGGGGCTGGACCTCGTGTTCGCCAACCGCCCCGACCACTCGGCTCC					
15	-MEXICO	GT CCGCTGCG CCGGG CTGGACCTCG TTCGCCAACC GCCCG CCACT GGTCC					
		5410v	5420v	5430v	5440v	5450v	5460v
20	-BURMA	GCTTGGCGTGACCAAGGCCAGCGCCCCGCGTTGCCTCAGCTCGTAGACCTACCACAGCT					
	-MEXICO	CTTGGCG GA CAGGCCAGCGCCCC CCG TGCCTC CGTCG GACCT CCACAGC					
		5470v	5480v	5490v	5500v	5510v	5520v
25	-BURMA	GGGGCCGCGCGCTAACCGCGGTGCTCCGGCCCATGACACCCGCCAGTGCTGATGTG					
	-MEXICO	GGGGC GCG CGCT AC GC GT GC CC GCCCATGACACC C CC GT CC GA GT					
		5530v	5540v	5550v	5560v	5570v	5580v
30	-BURMA	GACTCCCGCGGCGCATCTTGCGCGGCGAGTATAACCTATCAACATCTCCCTTACCTCT					
	-MEXICO	GA TC CGCGG GC AT T CGCGG CAGTATAA T TC AC TC CCCCT AC TC					
		5590v	5600v	5610v	5620v	5630v	5640v
35	-BURMA	TCCGTGGCCACCGGCACTAACCTGGTTCTTTATGCCGCCCTCTTAGTCCGCTTTTACCC					
	-MEXICO	TC GTGGCC C GGCACTAA T GT CT TATGC GCCCC CTTA TCCGC T T CC					
		5650v	5660v	5670v	5680v	5690v	5700v
40	-BURMA	CTTCAGGACGGCACCAATACCCATATAATGGCCACGGAAGCTTCTAATTATGCCAGTAC					
	-MEXICO	CT CAGGACGG AC AATAC CA AT ATGGCCAC GA GC TC AATTATGC CAGTAC					
		5710v	5720v	5730v	5740v	5750v	5760v
45	-BURMA	CGGGTTGCCCCGTGCCACAATCCGTTACCGCCCCGCTGGTCCCAATGCTGTGCGCGTTAC					
	-MEXICO	CGGGTTGCCCCG GC AC ATCCGTTACCG CC CT GT CC AATGC GT GG GG TA					
		5770v	5780v	5790v	5800v	5810v	5820v
50	-BURMA	GCCATCTCCATCTCATTCTGGCCACAGACCACCACCCCGACGTCGGTTGATATGAAT					
	-MEXICO	GC AT TCCAT TC TTCTGGCC CA AC ACCAC ACCCC AC TC GTTGA ATGAAT					

	5830v	5840v	5850v	5860v	5870v	5880v
-BURMA	TCAATAACCTCGACGGATGTTCTGATTTTAGTCCAGCCCGGCATAGCCTCTGAGCTTGTG					
-MEXICO	TC AT AC TC AC GATGT G ATT T GT CA CC GGCATAGC TCTGA T GT					
5						
	5890v	5900v	5910v	5920v	5930v	5940v
-BURMA	ATCCCAAGTGAGCGCCTACACTATCGTAACCAAGGCTGGCGCTCCGTCGAGACCTCTGGG					
-MEXICO	ATCCCAAG GAGCGCCT CACTA CG AA CAAGG TGGCGCTC GT GAGAC TCTGG					
10						
	5950v	5960v	5970v	5980v	5990v	6000v
-BURMA	GTGGCTGAGGAGGAGGCTACCTCTGGTCTTGTATGCTTTGCATACATGGCTCACTCGTA					
-MEXICO	GT GCTGAGGAGGA GC ACCTC GGTCTTGT ATG T TGCATACATGGCTC C GT					
15						
	6010v	6020v	6030v	6040v	6050v	6060v
-BURMA	AATTCCTATACTAATACACCCCTATACCGGTGCCCTCGGGCTGTTGGACTTTGCCCTTGAG					
-MEXICO	AA TCCTATAC AATAC CC TATACCGGTGCCCT GG T TGGACTTTGCC T GAG					
20						
	6070v	6080v	6090v	6100v	6110v	6120v
-BURMA	CTTGAGTTTCGCAACCTTACCCCGGTAACACCAATACGCGGGTCTCCCGTTATTCAGC					
-MEXICO	CTTGAGTTTCGCAA CT ACC CC GTAACACCAATAC CG GT TCCCGTTA TCCAGC					
25						
	6130v	6140v	6150v	6160v	6170v	6180v
-BURMA	ACTGCTCGCCACCGCCTTCGTGCGGGTGGGACGGGACTGCCGAGCTCACCACCACGGCT					
-MEXICO	ACTGCTCG CAC C CG G G GACGGGACTGC GAGCT ACCAC AC GC					
30						
	6190v	6200v	6210v	6220v	6230v	6240v
-BURMA	GCTACCCGCTTTATGAAGGACCTCTATTTTACTAGTACTAATGGTGTGCGGTGAGATCGGC					
-MEXICO	GC ACC G TT ATGAA GA CTC A TTTAC G TAATGG GT GGTGA TCGGC					
35						
	6250v	6260v	6270v	6280v	6290v	6300v
-BURMA	CGCGGGATAGCCCTCACCTGTTCAACCTTGCTGACACTCTGCTTGGCGGCCTGCCGACA					
-MEXICO	CGCGGGATAGC CT AC T T AACCTTGCTGACAC CT CT GGC GG CT CCGACA					
40						
	6310v	6320v	6330v	6340v	6350v	6360v
-BURMA	GAAATTGATTTCTGCTGGCTGGTGGCCAGCTGTTCTACTCCCGTCCCGTTGTCTCAGCCAAT					
-MEXICO	GAAATT ATTTCTGCTGGCTGG GG CA CTGTT TA TCCCG CC GTTGTCTCAGCCAAT					
45						
	6370v	6380v	6390v	6400v	6410v	6420v
-BURMA	GGCGAGCCGACTGTTAAGTTGTATACATCTGTAGAGAATGCTCAGCAGGATAAGGGTATT					
-MEXICO	GGCGAGCC AC GT AAG T TATACATC GT GAGAATGCTCAGCAGGATAAGGGT TT					
50						
	6430v	6440v	6450v	6460v	6470v	6480v
-BURMA	GCAATCCCGCATGACATTGACCTCGGAGAATCTCGTGTGGTTATTTCAGGATTATGATAAC					
-MEXICO	GC ATCCC CA GA AT GA CT GG GA TC CGTGTGGT ATTCAGGATTATGA AAC					
55						

		6490v	6500v	6510v	6520v	6530v	6540v
5	-BURMA	CAACATGAACAAGATCGGCCGACGCCTTCTCCAGCCCATCGCGCCTTTCTCTGTCCTT					
	-MEXICO	CA CATGA CA GATCGGCC AC CC TC CC GC CCATC CG CCTTT TCTGT CT					
		6550v	6560v	6570v	6580v	6590v	6600v
	-BURMA	CGAGCTAATGATGTGCTTTGGCTCTCTCACCCTGCCGAGTATGACCAGTCCACTTAT					
	-MEXICO	CGAGC AATGATGT CTTTGGCT TC CTCAC GC GCCGAGTATGACCAGTCCACTTA					
10		6610v	6620v	6630v	6640v	6650v	6660v
	-BURMA	GGCTCTTCGACTGGCCAGTTTATGTTTCTGACTCTGTGACCTTGGTTAATGTTGCGACC					
	-MEXICO	GG TC TC ACTGGCCC GTTTAT T TC GAC GTGAC TTGGT AATGTTGCGAC					
15		6670v	6680v	6690v	6700v	6710v	6720v
	-BURMA	GGCGCGCAGGCCGTTGCCCGGTCGCTCGATTGGACCAAGGTCACACTTGACGGTCGCCCC					
	-MEXICO	GGCGCGCAGGCCGT GCCG TCGCT GA TGG CCAA GTCAC CT GACGG CG CCC					
20		6730v	6740v	6750v	6760v	6770v	6780v
	-BURMA	CTCTCCACCATCCAGCAGTACTCGAAGACCTTCTTTGTCCTGCCGCTCCGCGTAAGCTC					
	-MEXICO	CTC C AC T AGCA TA TC AAGAC TTCTTGT CT CC CT CG GG AAGCTC					
25		6790v	6800v	6810v	6820v	6830v	6840v
	-BURMA	TCTTTCTGGGAGGCAGGCACAACATAAGCCGGGTACCCTTATAATTATAACCACTGCT					
	-MEXICO	TC TT TGGGAGGC GGCACAAC AAAGC GG TA CCTTATAATTATAA AC ACTGCT					
30		6850v	6860v	6870v	6880v	6890v	6900v
	-BURMA	AGCGACCAACTGCTTGTGCGAGAATGCCGCGGGCACC GGCTGCTATTCCACTTACACC					
	-MEXICO	AG GACCA T CT T GA AATGC GCCGG CA CGGGTCGC ATTTT AC TA ACC					
35		6910v	6920v	6930v	6940v	6950v	6960v
	-BURMA	ACTAGCCTGGGTGCTGGTCCCGTCTCCATTTCTGCGGTTGCCGTTTTAGCCCCCACTCT					
	-MEXICO	AC AG CT GG GC GGTCC GTC CCATTTCTGCGG GC GTTTT GC CC C CTC					
40		6970v	6980v	6990v	7000v	7010v	7020v
	-BURMA	GCGCTAGCATTGCTTGAGGATACCTTGGACTACCCTGCCCGCGCCATACTTTTGATGAT					
	-MEXICO	GC CT GC TGCT GAGGATAC TT GA TA CC G CG GC CA AC TTTGATGA					
45		7030v	7040v	7050v	7060v	7070v	7080v
	-BURMA	TTCTGCCAGAGTGCCGCCCTTGGCCTTCAGGGCTGCGCTTTCCAGTCTACTGTCGCT					
	-MEXICO	TTCTGCC GA TGCCGC C T GGCCT CAGGG TG GCTTTCCAGTC ACTGTCGCT					
50		7090v	7100v	7110v	7120v	7130v	7140v
	-BURMA	GAGCTTCAGCGCCTTAAGATGAAGGTGGGTAAAACCTCGGGAGTTGTAGTTTATTGCTTG					
	-MEXICO	GAGCT CAGCGCCTTAA T AAGGTGGGTAAAACCTCGGGAGTTGTAGTTTATTG TG					
55		7150v	7160v	7170v	7180v	7190v	7200v
	-BURMA	GAGCTCCAGCGCCTTAAAGTTAAGGTGGGTAAAACCTCGGGAGTTGTAGTTTATTGCTG					
	-MEXICO	GAGCTCCAGCGCCTTAAAGTTAAGGTGGGTAAAACCTCGGGAGTTGTAGTTTATTGCTG					

	7150v	7160v	7170v	7180v	7190v
-BURMA	TGCCCCCTTCTTTCTGTTC-----	TTATTTCTCATTTCTGCGTTCGCGCTCCC			
	TGCCC CCT CTT TGC	TTATTTT	TTTCT	GT	CCGCGCTCCC
-MEXICO	TGCCCACCTACTTATATCTGCTGATTTCTTTATTTCTTTTCTCGGTCCCGCGCTCCC				

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	v 7195
-BURMA	TGA
	TGA
-MEXICO	TGA

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A number of open reading frames, which are potential coding regions, have been found within the DNA sequences set forth above. As has already been noted, consensus residues for the RNA-directed RNA polymerase (RDRP) were identified in the HEV (Burma) strain clone ET1.1. Once a contiguous overlapping set of clones was accumulated, it became clear that the nonstructural elements containing the RDRP as well as what were identified as consensus residues for the helicase domain were located in the first large open reading frame (ORF1). ORF1 covers the 5' half of the genome and begins at the first encoded met, after the 27th bp of the apparent non-coding sequence, and then extends 5079 bp before reaching a termination codon. Beginning 37 bp downstream from the ORF1 stop codon in the plus 1 frame is the second major opening reading frame (ORF2) extending 1980 bp and terminating 68 bp upstream from the point of poly A addition. The third forward ORF (in the plus 2 frame) is also utilized by HEV. ORF3 is only 370 bp in length and would not have been predicted to be utilized by the virus were it not for the identification of the immunoreactive cDNA clone 406.4-2 from the Mexico SISPA cDNA library (see below for detailed discussion). This epitope confirmed the utilization of ORF3 by the virus, although the means by which this ORF is expressed has not yet been fully elucidated. If we assume that the first ~~met~~ is utilized, ORF3 overlaps ORF1 by 1 bp at its 5' end and ORF2 by 328 bp at its 3' end. ORF2 contains the broadly reactive 406.3-2 epitope and also

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a signal sequence at its extreme 5' end. The first half of this ORF2 also has a high pI value (>10) similar to that seen with other virus capsid proteins. These data suggest that the ORF2 might be the predominant structural gene of HEV.

The existence of subgenomic transcripts prompted a set of experiments to determine whether these RNAs were produced by splicing from the 5' end of the genome. An analysis using subgenomic probes from throughout the genome, including the extreme 5' end, did not provide evidence for a spliced transcript. However, it was discovered that a region of the genome displayed a high degree of homology with a 21 bp segment identified in Sindbis as a probably internal initiation site for RNA transcription used in the production of its subgenomic messages. Sixteen of 21 (76%) of the nucleotides are identical.

Two cDNA clones which encode an epitope of HEV that is recognized by sera collected from different ET-NANB outbreaks (i.e., a universally recognized epitope) have been isolated and characterized. One of the clones immunoreacted with 8 human sera from different infected individuals and the other clone immunoreacted with 7 of the human sera tested. Both clones immunoreacted specifically with cyno sera from infected animals and exhibited no immunologic response to sera from uninfected animals. The sequences of the cDNAs in these recombinant phages, designated 406.3-2 and 406.4-2 have been determined. The HEV open reading frames are shown to encode epitopes specifically recognized by sera from patients with HEV infections. The cDNA sequences and the polypeptides that they encode are set forth below.

Epitopes derived from Mexican strain of HEV:

406.4-2 sequence (nucleotide sequence has SEQ ID NO.13; amino acid sequence has SEQ ID NO.14):

SEQ ID NO. 13:

5	C GCC AAC CAG CCC GGC CAC TTG GCT CCA CTT GGC GAG ATC AGG CCC Ala Asn Gln Pro Gly His Leu Ala Pro Leu Gly Glu Ile Arg Pro	46
	1 5 10 15	
10	AGC GCC CCT CCG CTG CCT CCC GTC GCC GAC CTG CCA CAG CCG GGG CTG Ser Ala Pro Pro Leu Pro Pro Val Ala Asp Leu Pro Gln Pro Gly Leu	94
	20 25 30	
15	CGG CGC TGA CGGCTGTGGC GCCTGCCCAT GACACCTCAC CCGTCCCGGA Arg Arg .	143
20	CGTTGATTCT CGCGGTGCAA TTCTACGCCG CCAGTATAAT TTGTCTACTT CACCCCTGAC ATCCTCTGTG GCCTCTGGCA CTAATTTAGT CCTGTATGCA GCCCCCTTA ATCCGCCTCT GCCGCTGCAG GACGGTACTA ATACTCACAT TATGGCCACA GAGGCCTCCA ATTATGCACA GTACCGGGTT GCCCGCGCTA CTATCCGTTA CCGGCCCTA GTGCCTAATG CAGTTGGAGG CTATGCTATA TCCATTTCTT TCTGGCCTCA AACAACCACA ACCCCTACAT CTGTTGACAT GAATTC	203 263 323 383 443 449

SEQ ID NO. 14:

30	Ala Asn Gln Pro Gly His Leu Ala Pro Leu Gly Glu Ile Arg Pro Ser 1 5 10 15 Ala Pro Pro Leu Pro Pro Val Ala Asp Leu Pro Gln Pro Gly Leu Arg 20 25 30 Arg .
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406.3-2 sequence (nucleotide sequence has SEQ ID NO.15; amino acid sequence has SEQ ID NO.16):

SEQ ID NO. 15:

40	GGAT ACT TTT GAT TAT CCG GGG CGG GCG CAC ACA TTT GAT GAC TTC TGC Thr Phe Asp Tyr Pro Gly Arg Ala His Thr Phe Asp Asp Phe Cys 1 5 10 15	49
45	CCT GAA TGC CGC GCT TTA GGC CTC CAG GGT TGT GCT TTC CAG TCA ACT Pro Glu Cys Arg Ala Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr 20 25 30	97
50	GTC GCT GAG CTC CAG CGC CTT AAA GTT AAG GTT Val Ala Glu Leu Gln Arg Leu Lys Val Lys Val 35 40	130

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other sources of genetic material), as is well known in the art.

3. Two amino acid sequences or two nucleotide sequences (in an alternative definition for homology between two nucleotide sequences) are considered homologous (as this term is preferably used in this specification) if they have an alignment score of >5 (in standard deviation units) using the program ALIGN with the mutation gap matrix and a gap penalty of 6 or greater. See Dayhoff, M.O., in Atlas of Protein Sequence and Structure (1972) Vol. 5, National Biomedical Research Foundation, pp. 101-110, and Supplement 2 to this volume, pp. 1-10. The two sequences (or parts thereof, preferably at least 30 amino acids in length) are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program mentioned above.

4. A DNA fragment is "derived from" an ET-NANB viral agent if it has the same or substantially the same basepair sequence as a region of the viral agent genome.

5. A protein is "derived from" an ET-NANB viral agent if it is encoded by an open reading frame of a DNA or RNA fragment derived from an ET-NANB viral agent.

II. Obtaining Cloned ET-NANB Fragments

According to one aspect of the invention, it has been found that a virus-specific DNA clone can be produced by (a) isolating RNA from the bile of a cynomolgus monkey having a known ET-NANB infection, (b) cloning the cDNA fragments to form a fragment library, and (c) screening the library by differential hybridization to radiolabeled cDNAs from infected and non-infected bile sources.

A. cDNA Fragment Mixture

ET-NANB infection in cynomolgus monkeys is initiated by inoculating the animals intravenously with a 10% w/v suspension from human case stools positive for 27-34 nm ET-NANB particles (mean diameter 32 nm). An infected animal is monitored for elevated levels of alanine aminotransferase, indicating hepatitis infection. ET-NANB infection is confirmed by immunospecific binding of seropositive antibodies to virus-like particles (VLPs), according to published methods (Gravelle). Briefly, a stool (or bile) specimen taken from the infected animal 3-4 weeks after infection is diluted 1:10 with phosphate-buffered saline, and the 10x suspension is clarified by low-speed centrifugation and filtration successively through 1.2 and 0.45 micron filters. The material may be further purified by pelleting through a 30% sucrose cushion (Bradley). The resulting preparation of VLPs is mixed with diluted serum from human patients with known ET-NANB infection. After incubation overnight, the mixture is centrifuged overnight to pellet immune aggregates, and these are stained and examined by electron microscopy for antibody binding to the VLPs.

ET-NANB infection can also be confirmed by seroconversion to VLP-positive serum. Here the serum of the infected animal is mixed as above with 27-34 nm VLPs isolated from the stool specimens of infected human cases and examined by immune electron microscopy for antibody binding to the VLPs.

Bile can be collected from ET-NANB positive animals by either cannulating the bile duct and collecting the bile fluid or by draining the bile duct during necropsy. Total RNA is extracted from the bile by hot phenol extraction, as outlined in Example 1A. The RNA fragments are used to synthesize corresponding duplex cDNA fragments by random priming, also as referenced in Example 1A. The cDNA fragments may be fractionated by gel electrophoresis or density

gradient centrifugation to obtain a desired size class of fragments, e.g., 500-4,000 basepair fragments.

Although alternative sources of viral material, such as VLPs obtained from stool samples (as described in Example 4), may be used for producing a CDNA fraction, the bile source is preferred. According to one aspect of the invention, it has been found that bile from ET-NANB-infected monkeys shows a greater number of intact viral particles than material obtained from stool samples, as evidenced by immune electron microscopy. Bile obtained from an ET-NANB infected human or cynomolgus macaque, for use as a source of ET-NANB viral protein or genomic material, or intact virus, forms part of the present invention.

B. cDNA Library and Screening

The cDNA fragments from above are cloned into a suitable cloning vector to form a cDNA library. This may be done by equipping blunt-ended fragments with a suitable end linker, such as an EcoRI sequence, and inserting the fragments into a suitable insertion site of a cloning vector, such as at a unique EcoRI site. After initial cloning, the library may be re-cloned, if desired, to increase the percentage of vectors containing a fragment insert. The library construction described in Example 1B is illustrative. Here cDNA fragments were blunt-ended, equipped with EcoRI ends, and inserted into the EcoRI site of the lambda phage vector gt10. The library phage, which showed less than 5% fragment inserts, was isolated, and the fragment inserts re-cloned into the lambda gt10 vector, yielding more than 95% insert-containing phage.

The cDNA library is screened for sequences specific for ET-NANB by differential hybridization to cDNA probes derived from infected and non-infected sources. cDNA fragments from infected and non-infected source bile or stool viral isolates can be prepared as above. Radiolabeling the fragments is by random

labeling, nick translation, or end labeling,
according to conventional methods (Maniatis, p. 109).
The cDNA library from above is screened by transfer to
duplicate nitrocellulose filters, and hybridization
5 with both infected-source and non-infected-source
(control) radiolabeled probes, as detailed in Example
2. In order to recover sequences that hybridize at the
preferred outer limit of 25-30% basepair mismatches,
clones can be selected if they hybridize under the
10 conditions described in Maniatis et al., op. cit., pp.
320-323, but using the following wash conditions: 2 x
SCC, 0.1% SDS, room temperature - twice, 30 minutes
each; then 2 x SCC, 0.1% SDS, 50°C - once, 30 minutes;
then 2 x SCC, room temperature - twice, 10 minutes
15 each. These conditions allowed identification of the
Mexican isolate discussed above using the ET1.1
sequence as a probe. Plaques which show selective
hybridization to the infected-source probes are
preferably re-plated at low plating density and re-
20 screened as above, to isolate single clones which are
specific for ET-NANB sequences. As indicated in
Example 2, sixteen clones which hybridized
specifically with infected-source probes were
identified by these procedures. One of the clones,
25 designated lambda gt101.1, contained a 1.33 kilobase
fragment insert.

C. ET-NANB Sequences

The basepair sequence of cloned regions of the
30 ET-NANB fragments from Part B are determined by
standard sequencing methods. In one illustrative
method, described in Example 3, the fragment insert
from the selected cloning vector is excised, isolated
by gel electrophoresis, and inserted into a cloning
35 vector whose basepair sequence on either side of the
insertion site is known. The particular vector
employed in Example 3 is a pTZKF1 vector shown at the
left in Figure 1. The ET-NANB fragment from the gt10-

1.1 phage was inserted at the unique EcoRI site of the pTZKF1 plasmid. Recombinants carrying the desired insert were identified by hybridization with the isolated 1.33 kilobase fragment, as described in Example 3. One selected plasmid, identified as pTZKF1 (ET1.1), gave the expected 1.33 kb fragment after vector digestion with EcoRI. E. coli strain BB4 infected with the pTZKF1(ET1.1) plasmid has been deposited with the American Type Culture Collection, Rockville, MD, and is identified by ATCC deposit number 67717.

The pTZKF1(ET1.1) plasmid is illustrated at the bottom in Figure 1. The fragment insert has 5' and 3' end regions denoted at A and C, respectively, and an intermediate region, denoted at B. The sequences in these regions were determined by standard dideoxy sequencing and were set forth in an earlier application in this series. The three short sequences (A, B, and C) are from the same insert strand. As will be seen in Example 3, the B-region sequence was actually determined from the opposite strand, so that the B region sequence shown above represents the complement of the sequence in the sequenced strand. The base numbers of the partial sequences are approximate.

Later work in the laboratory of the inventors identified the full sequence, set forth above. Fragments of this total sequence can readily be prepared using restriction endonucleases. Computer analysis of both the forward and reverse sequence has identified a number of cleavage sites.

III. ET-NANB Fragments

According to another aspect, the invention includes ET-NANB-specific fragments or probes which hybridize with ET-NANB genomic sequences or cDNA fragments derived therefrom. The fragments may include full-length cDNA fragments such as described in

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Section II, or may be derived from shorter sequence regions within cloned cDNA fragments. Shorter fragments can be prepared by enzymatic digestion of full-length fragments under conditions which yield
5 desired-sized fragments, as will be described in Section IV. Alternatively, the fragments can be produced by oligonucleotide synthetic methods, using sequences derived from the cDNA fragments. Methods or commercial services for producing selected-sequence
10 oligonucleotide fragments are available. Fragments are usually at least 12 nucleotides in length, preferably at least 14, 20, 30 or 50 nucleotides, when used as probes. Probes can be full length or less than 500, preferably less than 300 or 200, nucleotides
15 in length.

To confirm that a given ET-NANB fragment is in fact derived from the ET-NANB viral agent, the fragment can be shown to hybridize selectively with cDNA from infected sources. By way of illustration, to
20 confirm that the 1.33 kb fragment in the pTZKF1(ET1.1) plasmid is ET-NANB in origin, the fragment was excised from the pTZKF1(ET1.1) plasmid, purified, and radiolabeled by random labeling. The radiolabeled fragment was hybridized with fractionated cDNAs from
25 infected and non-infected sources to confirm that the probe reacts only with infected-source cDNAs. This method is illustrated in Example 4, where the above radiolabeled 1.33 kb fragment from pTZKF1(ET1.1) plasmid was examined for binding to cDNAs prepared
30 from infected and non-infected sources. The infected sources are (1) bile from a cynomolgus macaque infected with a strain of virus derived from stool samples from human patients from Burma with known ET-NANB infections and (2) a viral agent derived from the
35 stool sample of a human ET-NANB patient from Mexico. The cDNAs in each fragment mixture were first amplified by a linker/primer amplification method described in Example 4. Fragment separation was on

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agarose gel, followed by Southern blotting and then hybridization to bind the radiolabeled 1.33 kb fragment to the fractionated cDNAs. The lane containing cDNAs from the infected sources showed a smeared band of bound probe, as expected (cDNAs amplified by the linker/primer amplification method would be expected to have a broad range of sizes). No probe binding to the amplified cDNAs from the non-infected sources was observed. The results indicate that the 1.33 kb probe is specific for cDNA fragments associated with ET-NANB infection. This same type of study, using ET 1.1 as the probe, has demonstrated hybridization to ET-NANB samples collected from Tashkent, Somalia, Borneo and Pakistan. Secondly, the fact that the probe is specific for ET-NANB related sequences derived from different continents (Asia, Africa and North America) indicates the cloned ET-NANB Burma sequence (ET1.1) is derived from a common ET-NANB virus or virus class responsible for ET-NANB hepatitis infection worldwide.

In a related confirmatory study, probe binding to fractionated genomic fragments prepared from human or cynomolgus macaque genomic DNA (both infected and uninfected) was examined. No probe binding was observed to either genomic fraction, demonstrating that the ET-NANB fragment is not an endogenous human or cynomolgus genomic fragment and additionally demonstrating that HEV is an RNA virus.

Another confirmation of ET NANB specific sequences in the fragments is the ability to express ET-NANB proteins from coding regions in the fragments and to demonstrated specific sero-reactivity of these proteins with sera collected during documented outbreaks of ET-NANB. Section IV below discusses methods of protein expression using the fragments.

One important use of the ET-NANB-specific fragments is for identifying ET-NANB-derived cDNAs which contain additional sequence information. The

newly identified cDNAs, in turn, yield new fragment probes, allowing further iterations until the entire viral genome is identified and sequenced. Procedures for identifying additional ET-NANB library clones and
5 generating new probes therefrom generally follow the cloning and selection procedures described in Section II.

The fragments (and oligonucleotides prepared based on the sequences given above) are also useful as
10 primers for a polymerase chain reaction method of detecting ET-NANB viral genomic material in a patient sample. This diagnostic method will be described in Section V below.

Two specific genetic sequences derived from
15 the Mexican strain, identified herein as 406.3-2 and 406.4-2, have been identified that encode immunogenic epitopes. This was done by isolating clones which encode epitopes that immunologically react specifically with sera from individuals and
20 experimental animals infected with HEV. Comparison of the isolated sequences with those in the Genbank collection of genetic sequences indicate that these viral sequences are novel. Since these sequences are unique, they can be used to identify the presence of
25 HEV and to distinguish this strain of hepatitis from HAV, HBV, and HCV strains. The sequences are also useful for the design of oligonucleotide probes to diagnose the presence of virus in samples. They can be used for the synthesis of polypeptides that
30 themselves are used in immunoassays. The specific 406.3-2 and 406.4-2 sequences can be incorporated into other genetic material, such as vectors, for ease of expression or replication. They can also be used (as demonstrated above) for identifying similar antigenic
35 regions encoded by related viral strains, such as the Burmese strain.

IV. ET-NANB Proteins

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As indicated above, ET-NANB proteins can be prepared by expressing open reading-frame coding regions in ET-NANB fragments. In one preferred approach, the ET-NANB fragments used for protein expression are derived from cloned cDNAs which have been treated to produce desired-size fragments, and preferably random fragments with sizes predominantly between about 100 to about 300 base pairs. Example 5 describes the preparation of such fragments by DNAs digestion. Because it is desired to obtain peptide antigens of between about 30 to about 100 amino acids, the digest fragments are preferably size fractionated, for example by gel electrophoresis, to select those in the approximately 100-300 basepair size range. Alternatively, cDNA libraries constructed directly from HEV-containing sources (e.g., bile or stool) can be screened directly if cloned into an appropriate expression vector (see below).

For example, the ET-NANB proteins expressed by the 406.3-2 and 406.4-2 sequences (and peptide fragments thereof) are particularly preferred since these proteins have been demonstrated to be immunoreactive with a variety of different human sera, thereby indicating the presence of one or more epitopes specific for HEV on their surfaces. These clones were identified by direct screening of a gt11 library.

A. Expression Vector

The ET-NANB fragments are inserted into a suitable expression vector. One exemplary expression vector is lambda gt11, which contains a unique EcoRI insertion site 53 base pairs upstream of the translation termination codon of the beta-galactosidase gene. Thus, the inserted sequence will be expressed as a beta-galactosidase fusion protein which contains the N-terminal portion of the beta-galactosidase gene, the heterologous peptide, and

optionally the C-terminal region of the beta-galactosidase peptide (the C-terminal portion being expressed when the heterologous peptide coding sequence does not contain a translation termination codon). This vector also produces a temperature-sensitive repressor (cl857) which causes viral lysogeny at permissive temperatures, e.g., 32°C, and leads to viral lysis at elevated temperatures, e.g., 37°C. Advantages of this vector include: (1) highly efficient recombinant generation, (2) ability to select lysogenized host cells on the basis of host-cell growth at permissive, but not non-permissive, temperatures, and (3) high levels of recombinant fusion protein production. Further, since phage containing a heterologous insert produces an inactive beta-galactosidase enzyme, phage with inserts can be readily identified by a beta-galactosidase colored-substrate reaction.

For insertion into the expression vector, the viral digest fragments may be modified, if needed, to contain selected restriction-site linkers, such as EcoRI linkers, according to conventional procedures. Example 1 illustrates methods for cloning the digest fragments into lambda gt11, which includes the steps of blunt-ending the fragments, ligating with EcoRI linkers, and introducing the fragments into EcoRI-cut lambda gt11. The resulting viral genomic library may be checked to confirm that a relatively large (representative) library has been produced. This can be done, in the case of the lambda gt11 vector, by infecting a suitable bacterial host, plating the bacteria, and examining the plaques for loss of beta-galactosidase activity. Using the procedures described in Example 1, about 50% of the plaques showed loss of enzyme activity.

B. Peptide Antigen Expression

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The viral genomic library formed above is screened for production of peptide antigen (expressed as a fusion protein) which is immunoreactive with antiserum from ET-NANB seropositive individuals . In a preferred screening method, host cells infected with phage library vectors are plated, as above, and the plate is blotted with a nitrocellulose filter to transfer recombinant protein antigens produced by the cells onto the filter. The filter is then reacted with the ET-NANB antiserum, washed to remove unbound antibody, and reacted with reporter-labeled, anti-human antibody, which becomes bound to the filter, in sandwich fashion, through the anti-ET-NANB antibody.

Typically phage plaques which are identified by virtue of their production of recombinant antigen of interest are re-examined at a relatively low density for production of antibody-reactive fusion protein. Several recombinant phage clones which produced immunoreactive recombinant antigen were identified in the procedure.

The selected expression vectors may be used for scale-up production, for purposes of recombinant protein purification. Scale-up production is carried out using one of a variety of reported methods for (a) lysogenizing a suitable host, such as E. coli, with a selected lambda gt11 recombinant (b) culturing the transduced cells under conditions that yield high levels of the heterologous peptide, and (c) purifying the recombinant antigen from the lysed cells.

In one preferred method involving the above lambda gt11 cloning vector, a high-producer E. coli host, BNN103, is infected with the selected library phage and replica plated on two plates. One of the plates is grown at 32°C, at which viral lysogeny can occur, and the other at 42°C, at which the infecting phage is in a lytic stage and therefore prevents cell growth. Cells which grow at the lower but not the

higher temperature are therefore assumed to be successfully lysogenized.

The lysogenized host cells are then grown under liquid culture conditions which favor high production of the fused protein containing the viral insert, and lysed by rapid freezing to release the desired fusion protein.

C. Peptide Purification

The recombinant peptide can be purified by standard protein purification procedures which may include differential precipitation, molecular sieve chromatography, ion-exchange chromatography, isoelectric focusing, gel electrophoresis and affinity chromatography. In the case of a fused protein, such as the beta-galactosidase fused protein prepared as above, the protein isolation techniques which are used can be adapted from those used in isolation of the native protein. Thus, for isolation of a soluble betagalactosidase fusion protein, the protein can be isolated readily by simple affinity chromatography, by passing the cell lysis material over a solid support having surface-bound anti-beta-galactosidase antibody.

D. Viral Proteins

The ET-NANB protein of the invention may also be derived directly from the ET-NANB viral agent. VLPs or protein isolated from stool or liver samples from an infected individual, as above, are one suitable source of viral protein material. The VLPs isolated from the stool sample may be further purified by affinity chromatography prior to protein isolation (see below). The viral agent may also be raised in cell culture, which provides a convenient and potentially concentrated source of viral protein. Co-owned U.S. Patent Application Serial No. 846,757, filed April 1, 1986, describes an immortalized trioma

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liver cell which supports NANB infection in cell culture. The trioma cell line is prepared by fusing human liver cells with a mouse/human fusion partner selected for human chromosome stability. Cells
5 containing the desired NANB viral agent can be identified by immunofluorescence methods, employing anti-ET-NANB human antibodies.

The viral agent is disrupted, prior to protein isolation, by conventional methods, which can
10 include sonication, high- or low-salt conditions, or use of detergents.

Purification of ET-NANB viral protein can be carried out by affinity chromatography, using a purified anti-ET-NANB antibody attached according to
15 standard methods to a suitable solid support. The antibody itself may be purified by affinity chromatography, where an immunoreactive recombinant ETNANB protein, such as described above, is attached to a solid support, for isolation of anti-ET-NANB
20 antibodies from an immune serum source. The bound antibody is released from the support by standard methods.

Alternatively, the anti-ET-NANB antibody may be an antiserum or a monoclonal antibody (Mab)
25 prepared by immunizing a mouse or other animal with recombinant ETNANB protein. For Mab production, lymphocytes are isolated from the animal and immortalized with a suitable fusion partner, and successful fusion products which react with the
30 recombinant protein immunogen are selected. These in turn may be used in affinity purification procedures, described above, to obtain native ET-NANB antigen.

V. Utility

35 Although ET-NANB is primarily of interest because of its effects on humans, recent data has shown that this virus is also capable of infecting other animals, especially mammals. Accordingly, any

discussion herein of utility applies to both human and veterinary uses, especially commercial veterinary uses, such as the diagnosis and treatment of pigs, cattle, sheep, horses, and other domesticated animals.

5 A. Diagnostic Methods

 The particles and antigens of the invention, as well as the genetic material, can be used in diagnostic assays. Methods for detecting the presence of ET-NANB hepatitis comprise analyzing a biological
10 sample such as a blood sample, stool sample or liver biopsy specimen for the presence of an analyte associated with ET-NANB hepatitis virus.

 The analyte can be a nucleotide sequence which hybridizes with a probe comprising a sequence of
15 at least about 16 consecutive nucleotides, usually 30 to 200 nucleotides, up to substantially the full sequence of the sequences shown above (cDNA sequences). The analyte can be RNA or cDNA. The analyte is typically a virus particle suspected of
20 being ET-NANB or a particle for which this classification is being ruled out. The virus particle can be further characterized as having an RNA viral genome comprising a sequence at least about 70% homologous to a sequence of at least 12 consecutive
25 nucleotides of the "forward" and "reverse" sequences given above, usually at least about 80% homologous to at least about 60 consecutive nucleotides within the sequences, and may comprise a sequence substantially homologous to the full-length sequences. In order to
30 detect an analyte, where the analyte hybridizes to a probe, the probe may contain a detectable label. Particularly preferred for use as a probe are sequences of consecutive nucleotides derived from the 406.3-2 and 406.4-2 clones described herein, since
35 these clones appear to be particularly diagnostic for HEV.

 The analyte can also comprise an antibody which recognizes an antigen, such as a cell surface

antigen, on a ET-NANB virus particle. The analyte can also be a ET-NANB viral antigen. Where the analyte is an antibody or an antigen, either a labelled antigen or antibody, respectively, can be used to bind to the
5 analyte to form an immunological complex, which can then be detected by means of the label.

Typically, methods for detecting analytes such as surface antigens and/or whole particles are based on immunoassays. Immunoassays can be conducted
10 either to determine the presence of antibodies in the host that have arisen from infection by ET-NANB hepatitis virus or by assays that directly determine the presence of virus particles or antigens. Such techniques are well known and need not be described
15 here in detail. Examples include both heterogeneous and homogeneous immunoassay techniques. Both techniques are based on the formation of an immunological complex between the virus particle or its antigen and a corresponding specific antibody.
20 Heterogeneous assays for viral antigens typically use a specific monoclonal or polyclonal antibody bound to a solid surface. Sandwich assays are becoming increasingly popular. Homogeneous assays, which are carried out in solution without the presence of a
25 solid phase, can also be used, for example by determining the difference in enzyme activity brought on by binding of free antibody to an enzyme-antigen conjugate. A number of suitable assays are disclosed in U.S. Patent Nos. 3,817,837, 4,006,360, 3,996,345.

30 When assaying for the presence of antibodies induced by ET-NANB viruses, the viruses and antigens of the invention can be used as specific binding agents to detect either IgG or IgM antibodies. Since IgM antibodies are typically the first antibodies that
35 appear during the course of an infection, when IgG synthesis may not yet have been initiated, specifically distinguishing between IgM and IgG antibodies present in the blood stream of a host will

enable a physician or other investigator to determine whether the infection is recent or convalescent. Proteins expressed by the 406.3-2 and 406.4-2 clones described herein and peptide fragments thereof are particularly preferred for use as specific binding agents to detect antibodies since they have been demonstrated to be reactive with a number of different human HEV sera. Further, they are reactive with both acute and convalescent sera.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having surface-bound ET-NANB protein antigen. After binding anti-ET-NANB antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-ET-NANB antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric or colorimetric substrate.

The solid surface reagent in the above assay prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activate carboxyl, hydroxyl, or aldehyde group.

In a second diagnostic configuration, known as a homogeneous assay, antibody binding to a solid support produces some change in the reaction medium which can be directly detected in the medium. Known general types of homogeneous assays proposed

heretofore include (a) spin-labeled reporters, where antibody binding to the antigen is detected by a change in reported mobility (broadening of the spin splitting peaks), (b) fluorescent reporters, where
5 binding is detected by a change in fluorescence efficiency, (c) enzyme reporters, where antibody binding effects enzyme/substrate interactions, and (d) liposome-bound reporters, where binding leads to liposome lysis and release of encapsulated reporter.
10 The adaptation of these methods to the protein antigen of the present invention follows conventional methods for preparing homogeneous assay reagents.

In each of the assays described above, the assay method involves reacting the serum from a test
15 individual with the protein antigen and examining the antigen for the presence of bound antibody. The examining may involve attaching a labeled anti-human antibody to the antibody being examined, either IgM (acute phase) or IgG (convalescent phase), and
20 measuring the amount of reporter bound to the solid support, as in the first method, or may involve observing the effect of antibody binding on a homogeneous assay reagent, as in the second method.

Also forming part of the invention is an
25 assay system or kit for carrying out the assay method just described. The kit generally includes a support with surface-bound recombinant protein antigen which is (a) immunoreactive with antibodies present in individuals infected with enterically transmitted
30 nonA/nonB viral agent and (b) derived from a viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. Coli strain BB4, and having ATCC deposit no. 67717. A reporter-labeled anti-human antibody in the kit is used for
35 detecting surface-bound anti-ET-NANB antibody.

B. Viral Genome Diagnostic Applications

The genetic material of the invention can itself be used in numerous assays as probes for genetic material present in naturally occurring infections. One method for amplification of target nucleic acids, for later analysis by hybridization assays, is known as the polymerase chain reaction or PCR technique. The PCR technique can be applied to detecting virus particles of the invention in suspected pathological samples using oligonucleotide primers spaced apart from each other and based on the genetic sequence set forth above. The primers are complementary to opposite strands of a double stranded DNA molecule and are typically separated by from about 50 to 450 nt or more (usually not more than 2000 nt). This method entails preparing the specific oligonucleotide primers and then repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. Extension products generated from one primer serve as additional target sequences for the other primer. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2^n where n is the number of cycles. Given that the average efficiency per cycle ranges from about 65% to 85%, 25 cycles produce from 0.3 to 4.8 million copies of the target sequence. The PCR method is described in a number of publications, including Saiki *et al.*, Science (1985) 230:1350-1354; Saiki *et al.*, Nature (1986) 324:163-166; and Scharf *et al.*, Science (1986) 233:1076-1078. Also see U.S. Patent Nos. 4,683,194; 4,683,195; and 4,683,202.

The invention includes a specific diagnostic method for determination of ET-NANB viral agent, based on selective amplification of ET-NANB fragments. This method employs a pair of single-strand primers derived

from non-homologous regions of opposite strands of a DNA duplex fragment, which in turn is derived from an enterically transmitted viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4, and having ATCC deposit no. 67717. These "primer fragments," which form one aspect of the invention, are prepared from ET-NANB fragments such as described in Section III above. The method follows the process for amplifying selected nucleic acid sequences as disclosed in U.S. Patent No. 4,683,202, as discussed above.

15 C. Peptide Vaccine

Any of the antigens of the invention can be used in preparation of a vaccine. A preferred starting material for preparation of a vaccine is the particle antigen isolated from bile. The antigens are preferably initially recovered as intact particles as described above. However, it is also possible to prepare a suitable vaccine from particles isolated from other sources or non-particle recombinant antigens. When non-particle antigens are used (typically soluble antigens), proteins derived from the viral envelope or viral capsid are preferred for use in preparing vaccines. These proteins can be purified by affinity chromatography, also described above.

If the purified protein is not immunogenic per se, it can be bound to a carrier to make the protein immunogenic. Carriers include bovine serum albumin, keyhole limpet hemocyanin and the like. It is desirable, but not necessary, to purify antigens to be substantially free of human protein. However, it is more important that the antigens be free of proteins, viruses, and other substances not of human origin that may have been introduced by way of, or contamination of, the nutrient medium, cell lines,

tissues, or pathological fluids from which the virus is cultured or obtained.

Vaccination can be conducted in conventional fashion. For example, the antigen, whether a viral particle or a protein, can be used in a suitable diluent such as water, saline, buffered salines, complete or incomplete adjuvants, and the like. The immunogen is administered using standard techniques for antibody induction, such as by subcutaneous administration of physiologically compatible, sterile solutions containing inactivated or attenuated virus particles or antigens. An immune response producing amount of virus particles is typically administered per vaccinizing injection, typically in a volume of one milliliter or less.

A specific example of a vaccine composition includes, in a pharmacologically acceptable adjuvant, a recombinant protein or protein mixture derived from an enterically transmitted nonA/nonB viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4, and having ATCC deposit no. 67717. The vaccine is administered at periodic intervals until a significant titer of anti-ET-NANB antibody is detected in the serum. The vaccine is intended to protect against ET-NANB infection.

Particularly preferred are vaccines prepared using proteins expressed by the 406.3-2 and 406.4-2 clones described herein and equivalents thereof, including fragments of the expressed proteins. Since these clones have already been demonstrated to be reactive with a variety of human HEV-positive sera, their utility in protecting against a variety of HEV strains is indicated.

D. Prophylactic and Therapeutic
Antibodies and Antisera

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In addition to use as a vaccine, the compositions can be used to prepare antibodies to ET-NANB virus particles. The antibodies can be used directly as antiviral agents. To prepare antibodies, a host animal is immunized using the virus particles or, as appropriate, non-particle antigens native to the virus particle are bound to a carrier as described above for vaccines. The host serum or plasma is collected following an appropriate time interval to provide a composition comprising antibodies reactive with the virus particle. The gamma globulin fraction or the IgG antibodies can be obtained, for example, by use of saturated ammonium sulfate or DEAE Sephadex, or other techniques known to those skilled in the art. The antibodies are substantially free of many of the adverse side effects which may be associated with other anti-viral agents such as drugs.

The antibody compositions can be made even more compatible with the host system by minimizing potential adverse immune system responses. This is accomplished by removing all or a portion of the FC portion of a foreign species antibody or using an antibody of the same species as the host animal, for example, the use of antibodies from human/human hybridomas.

The antibodies can also be used as a means of enhancing the immune response since antibody-virus complexes are recognized by macrophages. The antibodies can be administered in amounts similar to those used for other therapeutic administrations of antibody. For example, pooled gamma globulin is administered at 0.02-0.1 ml/lb body weight during the early incubation of other viral diseases such as rabies, measles and hepatitis B to interfere with viral entry into cells. Thus, antibodies reactive with the ET-NANB virus particle can be passively administered alone or in conjunction with another anti-viral agent to a host infected with an ET-NANB virus to enhance the immune

response and/or the effectiveness of an antiviral drug.

Alternatively, anti-ET-NANB-virus antibodies can be induced by administering anti-idiotypic antibodies as immunogens. Conveniently, a purified anti-ET-NANB-virus antibody preparation prepared as described above is used to induce anti-idiotypic antibody in a host animal. The composition is administered to the host animal in a suitable diluent. Following administration, usually repeated administration, the host produces anti-idiotypic antibody. To eliminate an immunogenic response to the Fc region, antibodies produced by the same species as the host animal can be used or the Fc region of the administered antibodies can be removed. Following induction of anti-idiotypic antibody in the host animal, serum or plasma is removed to provide an antibody composition. The composition can be purified as described above for anti-ET-NANB virus antibodies, or by affinity chromatography using anti-ET-NANB-virus antibodies bound to the affinity matrix. The anti-idiotypic antibodies produced are similar in conformation to the authentic ET-NANB antigen and may be used to prepare an ET-NANB vaccine rather than using a ET-NANB particle antigen.

When used as a means of inducing anti-ET-NANB virus antibodies in a patient, the manner of injecting the antibody is the same as for vaccination purposes, namely intramuscularly, intraperitoneally, subcutaneously or the like in an effective concentration in a physiologically suitable diluent with or without adjuvant. One or more booster injections may be desirable. The anti-idiotypic method of induction of anti-ET-NANB virus antibodies can alleviate problems which may be caused by passive administration of anti-ET-NANB-virus antibodies, such as an adverse immune response, and those associated

with administration of purified blood components, such as infection with as yet undiscovered viruses.

5 The ET-NANB derived proteins of the invention are also intended for use in producing antiserum designed for pre- or post-exposure prophylaxis. Here an ET-NANB protein, or mixture of proteins is formulated with a suitable adjuvant and administered by injection to human volunteers, according to known methods for producing human antisera. Antibody response to the
10 injected proteins is monitored, during a several- week period following immunization, by periodic serum sampling to detect the presence an anti-ET-NANB serum antibodies, as described in Section IIA above.

15 The antiserum from immunized individuals may be administered as a pre-exposure prophylactic measure for individuals who are at risk of contracting infection. The antiserum is also useful in treating an individual post-exposure, analogous to the use of high titer antiserum against hepatitis B virus for post-
20 exposure prophylaxis.

E. Monoclonal Antibodies

For both in vivo use of antibodies to ET-NANB virus particles and proteins and anti-idiotypic
25 antibodies and diagnostic use, it may be preferable to use monoclonal antibodies. Monoclonal anti-virus particle antibodies or anti-idiotypic antibodies can be produced as follows. The spleen or lymphocytes from an immunized animal are removed and immortalized or used
30 to prepare hybridomas by methods known to those skilled in the art. To produce a human-human hybridoma, a human lymphocyte donor is selected. A donor known to be infected with a ET-NANB virus (where infection has been shown for example by the presence
35 of anti-virus antibodies in the blood or by virus culture) may serve as a suitable lymphocyte donor. Lymphocytes can be isolated from a peripheral blood sample or spleen cells may be used if the donor is

subject to splenectomy. Epstein-Barr virus (EBV) can be used to immortalize human lymphocytes or a human fusion partner can be used to produce human-human hybridomas. Primary in vitro immunization with peptides can also be used in the generation of human monoclonal antibodies.

Antibodies secreted by the immortalized cells are screened to determine the clones that secrete antibodies of the desired specificity. For monoclonal anti-virus particle antibodies, the antibodies must bind to ET-NANB virus particles. For monoclonal anti-idiotypic antibodies, the antibodies must bind to anti-virus particle antibodies. Cells producing antibodies of the desired specificity are selected.

The following examples illustrate various aspects of the invention, but are in no way intended to limit the scope thereof.

Material

The materials used in the following Examples were as follows:

Enzymes: DNase I and alkaline phosphatase were obtained from Boehringer Mannheim Biochemicals (BMB, Indianapolis, IN); EcoRI, EcoRI methylase, DNA ligase, and DNA Polymerase I, from New England Biolabs (NEB, Beverly MA); and RNase A was obtained from Sigma (St. Louis, MO) .

Other reagents: EcoRI linkers were obtained from NEB; and nitro blue tetrazolium (NBT), S-bromo-4-chloro-3-indolyl phosphate (BCIP) S-bromo-4-chloro-3-indolyl-B-D-galactopyranoside (Xgal) and isopropyl B-D-thiogalactopyranoside (IPTG) were obtained from Sigma.

cDNA synthesis kit and random priming labeling kits are available from Boehringer-Mannheim Biochemical (BMB, Indianapolis, IN).

Preparing cDNA Library

Two cynomolgus monkeys (cynos) were intravenously injected with a 10% suspension of a stool pool obtained from a second-passage cyno (cyno #37) infected with a strain of ET-NANB virus isolated from Burma cases whose stools were positive for ET-NANB, as evidenced by binding of 27-34 nm virus-like particles (VLPs) in the stool to immune serum from a known ETNANB patient. The animals developed elevated levels of alanine aminotransferase (ALT) between 24-36 days after inoculation, and one excreted 27-34 nm VLPs in its bile in the pre-acute phase of infection.

15 The bile duct of each infected animal was cannulated and about 1-3 cc of bile was collected daily. RNA was extracted from one bile specimen (cyno #121) by hot phenol extraction, using a standard RNA isolation procedure. Double-strand cDNA was formed
20 from the isolated RNA by a random primer for first-strand generation, using a cDNA synthesis kit obtained from Boehringer-Mannheim (Indianapolis, IN).

25 The duplex cDNA fragments were blunt-ended
with T4 DNA polymerase under standard conditions
(Maniatis, p. 118), then extracted with
phenol/chloroform and precipitated with ethanol. The
blunt-ended material was ligated with EcoRI linkers
30 under standard conditions (Maniatis, pp. 396-397) and
digested with EcoRI to remove redundant linker ends.
Non-ligated linkers were removed by sequential
isopropanol precipitation.

35 Lambda gt10 phage vector (Huynh) was
obtained from Promega Biotec (Madison, WI). This
cloning vector has a unique EcoRI cloning site in the
phage CI repressor gene. The cDNA fragments from above
were introduced into the EcoRI site by mixing 0.5 -

1.0 μ g EcoRI-cleaved gt10, 0.5-3 μ l of the above
duplex fragments, 0.5 μ l 10X ligation buffer, 0.5 μ l
ligase (200 units), and distilled water to 5 μ l. The
mixture was incubated overnight at 14°C, followed by
5 in vitro packaging, according to standard methods
(Maniatis, pp. 256-268).

The packaged phage were used to infect an E.
coli hfl strain, such as strain HG415. Alternatively,
E. coli, strain C600 hfl available from Promega
10 Biotec, Madison, WI, could be used. The percentage of
recombinant plaques obtained with insertion of the
EcoRI-ended fragments was less than 5% by analysis of
20 random plaques.

The resultant cDNA library was plated and
15 phage were eluted from the selection plates by
addition of elution buffer. After DNA extraction from
the phage, the DNA was digested with EcoRI to release
the heterogeneous insert population, and the DNA
fragments were fractionated on agarose to remove phage
20 fragments. The 500-4,000 basepair inserts were
isolated and recloned into lambda gt10 as above, and
the packaged phage was used to infect E. coli strain
HG415. The percentage of successful recombinants was
greater than 95%. The phage library was plated on E.
25 coli strain HG415, at about 5,000 plaques/plate, on a
total of 8 plates.

Example 2

Selecting ET-NANB Cloned Fragments

30 A. cDNA Probes

Duplex cDNA fragments from noninfected and
ETNANB-infected cynomolgus monkeys were prepared as in
Example 1. The cDNA fragments were radiolabeled by
random priming, using a random-priming labeling kit
35 obtained from Boehringer-Mannheim (Indianapolis, IN).

B. Clone Selection

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The plated cDNA library from Example 1 was transferred to each of two nitrocellulose filters, and the phage DNA was fixed on the filters by baking, according to standard methods (Maniatis, pp. 320323).
5 The duplicate filters were hybridized with either infected-source or control CDNA probes from above. Autoradiographs of the filters were examined to identify library clones which hybridized with radiolabeled CDNA probes from infected source only,
10 i.e., did not hybridize with cDNA probes from the non-infected source. Sixteen such clones, out of a total of about 40,000 clones examined, were identified by this subtraction selection method.

Each of the sixteen clones was picked and
15 replated at low concentration on an agar plate. The clones on each plate were transferred to two nitrocellulose ag duplicate lifts, and examined for hybridization to radiolabeled cDNA probes from infected and noninfected sources, as above. Clones were selected
20 which showed selective binding for infected-source probes (i.e., binding with infected-source probes and substantially no binding with non-infected-source probes). One of the clones which bound selectively to probe from infected source was isolated for further
25 study. The selected vector was identified as lambda gt10-1.1, indicated in Figure 1.

Example 3

ET-NANB Sequence

30 Clone lambda gt10-1.1 from Example 2 was digested with EcoRI to release the heterologous insert, which was separated from the vector fragments by gel electrophoresis. The electrophoretic mobility of the fragment was consistent with a 1.33 kb fragment. This
35 fragment, which contained EcoRI ends, was inserted into the EcoRI site of a pTZKF1 vector, whose construction and properties are described in co-owned U.S. patent application for "Cloning Vector System and

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Method for Rare Clone Identification", Serial No. 125, 650, filed November 25, 1987. Briefly, and as illustrated in Figure 1, this plasmid contains a unique EcoRI site adjacent a T7 polymerase promoter site, and plasmid and phage origins of replication. The sequence immediately adjacent each side of the EcoRI site is known. E. coli BB4 bacteria, obtained from Stratagene (La Jolla, CA, were transformed with the plasmid.

10 Radiolabeled ET-NANB probe was prepared by excising the 1.33 kb insert from the lambda gt10-1.1 phage in Example 2, separating the fragment by gel electrophoresis, and randomly labeling as above. Bacteria transfected with the above pTZKF1 and

15 containing the desired ET-NANB insert were selected by replica lift and hybridization with the radiolabeled ET-NANB probe, according to methods outlined in Example 2.

20 One bacterial colony containing a successful recombinant was used for sequencing a portion of the 1.33 kb insert. This isolate, designated pTZKF1(ET1.1), has been deposited with the American Type Culture Collection, and is identified by ATCC deposit no. 67717. Using a standard dideoxy

25 sequencing procedure, and primers for the sequences flanking the EcoRI site, about 200-250 basepairs of sequence from the 5'-end region and 3'-end region of the insert were obtained. The sequences are given above in Section II. Later sequencing by the same

30 techniques gave the full sequence in both directions, also given above.

Example 4

Detecting ET-NANB Sequences

35 cDNA fragment mixtures from the bile of noninfected and ET-NANB-infected cynomolgus monkeys were prepared as above. The cDNA fragments obtained from human stool samples were prepared as follows.

strand/primer complexes, and replication, was repeated 25 times.

The amplified cDNA sequences were fractionated by agarose gel electrophoresis, using a 2% agarose matrix. After transfer of the DNA fragments from the agarose gels to nitrocellulose paper, the filters were hybridized to a random-labeled 32p probe prepared by (i) treating the pTZKF1(ET1.1) plasmid from above with EcoRI, (ii) isolating the released 1.33 kb ET-NANB fragment, and (iii) randomly labeling the isolated fragment. The probe hybridization was performed by conventional Southern blotting methods (Maniatis, pp. 382-389). Figure 2 shows the hybridization pattern obtained with cDNAs from infected (I) and non-infected (N) bile sources (2A) and from infected (I) and noninfected (N) human stool sources (2B). As seen, the ET-NANB probe hybridized with fragments obtained from both of the infected sources, but was non-homologous to sequences obtained from either of the non-infected sources, thus confirming the specificity of derived sequence.

Southern blots of the radiolabeled 1.33 kb fragment with genomic DNA fragments from both human and cynomolgus-monkey DNA were also prepared. No probe hybridization to either of the genomic fragment mixtures was observed, confirming that the ET-NANB sequence is exogenous to either human or cynomolgus genome.

30 Example 5

Expressing ET-NANB Proteins

A. Preparing ET-NANB Coding Sequences

The pTZKF1(ET1.1) plasmid from Example 2 was digested with EcoRI to release the 1.33 kb ET-NANB insert which was purified from the linearized plasmid by gel electrophoresis. The purified fragment was suspended in a standard digest buffer (0.5M Tris HCl, pH 7.5; 1 mg/ml BSA; 10mM MnCl2) to a concentration of

about 1 mg/ml and digested with DNase I at room temperature for about 5 minutes. These reaction conditions were determined from a prior calibration study, in which the incubation time required to produce predominantly 100-300 basepair fragments was determined. The material was extracted with phenol/chloroform before ethanol precipitation.

The fragments in the digest mixture were blunt-ended and ligated with EcoRI linkers as in Example 1. The resultant fragments were analyzed by electrophoresis (5-10V/cm) on 1.2% agarose gel, using PhiX174/HaeIII and lambda/HindIII size markers. The 100-300 bp fraction was eluted onto NA45 strips (Schleicher and Schuell), which were then placed into 1.5 ml microtubes with eluting solution (1 M NaCl, 50 mM arginine, pH 9.0), and incubated at 67°C for 30-60 minutes. The eluted DNA was phenol/chloroform extracted and then precipitated with two volumes of ethanol. The pellet was resuspended in 20 μ l TE (0.01 M Tris HCl, pH 7.5, 0.001 M EDTA).

B. Cloning in an Expression Vector

Lambda gt11 phage vector (Huynh) was obtained from Promega Biotec (Madison, WI). This cloning vector has a unique EcoRI cloning site 53 base pairs upstream from the beta-galactosidase translation termination codon. The genomic fragments from above, provided either directly from coding sequences (Example 5) or after amplification of cDNA (Example 4), were introduced into the EcoRI site by mixing 0.5-1.0 μ g EcoRI-cleaved gt11, 0.3-3 μ l of the above sized fragments, 0.5 μ l 10X ligation buffer (above), 0.5 μ l ligase (200 units), and distilled water to 5 μ l. The mixture was incubated overnight at 14°C, followed by in vitro packaging, according to standard methods (Maniatis, pp. 256-268).

The packaged phage were used to infect E. coli strain KM392, obtained from Dr. Kevin Moore, DNAX

(Palo Alto, CA). Alternatively, E. Coli strain Y1090, available from the American Type Culture Collection (ATCC #37197), could be used. The infected bacteria were plated and the resultant colonies were checked for loss of beta-galactosidase activity-(clear plaques) in the presence of X-gal using a standard X-gal substrate plaque assay method (Maniatis). About 50% of the phage plaques showed loss of beta-galactosidase enzyme activity (recombinants).

C. Screening for ET-NANB Recombinant Proteins

ET-NANB convalescent antiserum was obtained from patients infected during documented ET-NANB outbreaks in Mexico, Borneo, Pakistan, Somalia, and Burma. The sera were immunoreactive with VLPs in stool specimens from each of several other patients with ET-NANB hepatitis.

A lawn of E. coli KM392 cells infected with about 104 pfu of the phage stock from above was prepared on a 150 mm plate and incubated, inverted, for 5-8 hours at 37°C. The lawn was overlaid with a nitrocellulose sheet, causing transfer of expressed ETNANB recombinant protein from the plaques to the paper. The plate and filter were indexed for matching corresponding plate and filter positions.

The filter was washed twice in TBST buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20), blocked with AIB (TBST buffer with 1% gelatin), washed again in TBST, and incubated overnight after addition of antiserum (diluted to 1:50 in AIB, 12-15 ml/plate). The sheet was washed twice in TBST and then contacted with enzyme-labeled anti-human antibody to attach the labeled antibody at filter sites containing antigen recognized by the antiserum. After a final washing, the filter was developed in a substrate medium containing 33 μ l NBT (50 mg/ml stock solution maintained at 4°C) mixed with 16 μ l BCIP (50 mg/ml stock solution maintained at 4°C) in 5 ml of alkaline

TABLE 1

	<u>Subclone</u>	<u>Position in "Reverse" Sequence</u>	
		<u>5'-end</u>	<u>3'-end</u>
5	Y1	522	643
	Y2	594	667
	Y3	508	665
	Y4	558	752
	Y5	545	665

Since all of the gene sequences identified in the table must contain the coding sequence for the epitope, it is apparent that the coding sequence for the epitope falls in the region between nucleotide 594 (5'-end) and 643 (3'-end). Genetic sequences equivalent to and complementary to this relatively short sequence are therefore particularly preferred aspects of the present invention, as are peptides produced using this coding region.

A second series of clones identifying an altogether different epitope was isolated with only Mexican serum.

TABLE 2

	<u>Subclone</u>	<u>Position in "Forward" Sequence</u>	
		<u>5'end</u>	<u>3' end</u>
30	ET 2-2	2	193
	ET 8-3	2	135
	ET 9-1	2	109
	ET 13-1	2	101

The coding system for this epitope falls between nucleotide 2 (5'-end) and 101 (3'-end). Genetic sequences related to this short sequence are therefore also preferred, as are peptides produced using this coding region.

Two particularly preferred subclones for use in preparing polypeptides containing epitopes specific for HEV are the 406.3-2 and 406.4-2 clones whose sequences are set forth above. These sequences were isolated from an amplified cDNA library derived from a Mexican stool. Using the techniques described in this section, polypeptides expressed by these clones have been tested for immunoreactivity against a number of different human HEV-positive sera obtained from sources around the world. As shown in Table 3 below, 8 sera immunoreactive with the polypeptide expressed by the 406.4-2, and 6 sera immunoreacted with polypeptide expressed by the 406.3-2 clone.

For comparison, the Table also shows reactivity of the various human sera with the Y2 clone identified in Table 1 above. Only one of the sera reacted with the polypeptide expressed by this clone. No immunoreactivity was seen for normal expression products of the gtl1 vector.

Table 3

Immunoreactivity of HEV Recombinant
Proteins: Human Sera

Sera	Source	Stage 1	406.3-2	406.4-2	Y2	λgt11
FVH-21	Burma	A'	-	-	-	-
FVH-8	Burma	A	-	+	+	-
SOM-19	Somalia	A	+	+	-	-
SOM-20	Somalia	A	+	+	-	-
IM-35	Borneo	A	+	+	-	-
IM-36	Borneo	A	-	-	-	-
PAK-1	Pakistan	A	+	+	-	-
FFI-4	Mexico	A	+	+	-	-

FFI-125	Mexico	A	-	+	-	-
F 387 IC	Mexico	C	+	+	ND	-
Normal	U.S.A.	-	-	-	-	-

5 1A = acute; C = convalescent

While the invention has been described with reference to particular embodiments, methods, construction and use, it will be apparent to those skilled in the art that various changes and

10 modifications can be made without departing from the invention.

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